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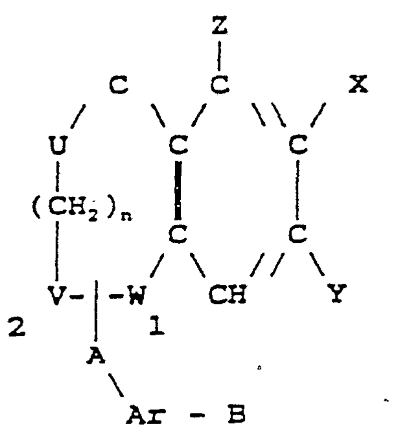

(Q)

(57) Abstract

Compounds of formula (Q), wherein X and Y form a five- or six-membered heterocyclic ring containing at least one nitrogen; Z is a hydrogen, halogen, carbon, oxygen or nitrogen atom; U is a carbon or nitrogen atom; n is 0 or the integer 1; V is a carbon or nitrogen atom; W is a carbon or nitrogen atom; A is in the 1- or 2-position and is a nitrogen atom, a sulfur atom, or a substituted or unsubstituted alkylene group; Ar is an aryl or heteroaryl group having one or more rings; and B is either (i) an oxygen or nitrogen atom, or a -CO- or -SO_2- group, any of which is linked to an amino acid, aryl group, heterocyclic group or alkyl group, or (ii) a substituted or unsubstituted alkyl group, which inhibit the enzyme thymidylate synthase ("TS"), pharmaceutical compositions containing these tricyclic compounds, and the use of these compounds to inhibit TS, including all effects derived from the inhibition of TS. Effects derived from the inhibition of TS include the inhibition of the growth and proliferation of the cells of higher organisms and of microorganisms, such as yeast and fungi. Such effects include antitumor activity.

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+ DESIGNATIONS OF "SU"

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DESCRIPTION

SUBSTITUTED TRICYCLIC COMPOUNDS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to certain substituted tricyclic compounds which inhibit the enzyme thymidylate synthase ("TS"), to pharmaceutical compositions containing these tricyclic compounds, and to the use of these compounds to inhibit TS, including all effects derived from the inhibition of TS. Effects derived from the inhibition of TS include the inhibition of the growth and proliferation of the cells of higher organisms and of microorganisms, such as yeast and fungi. Such effects include antitumor activity. A process for the preparation of the substituted tricyclic compounds of the invention is also disclosed.

The large class of antiproliferative agents includes antimetabolite compounds. A particular subclass of antimetabolites known as antifolates or "antifols" are antagonists of the vitamin folic acid. Typically, antifolates closely resemble the structure of folic acid, including the characteristic p-benzoyl glutamate moiety of folic acid. TS has long been considered an important target enzyme in the design and

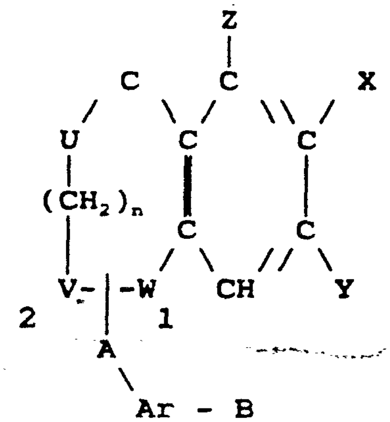
synthesis of antitumor agents, and a number of folate analogues have been synthesized and studied for their ability to inhibit TS. See, for example, Brixner et al., Folate Analogues as Inhibitors of Thymidylate Synthase, J. Med. Chem. 30, 675 (1987); Jones et al., Quinazoline Antifolates Inhibiting Thymidylate Synthase: Benzoyl Ring Modifications, J. Med. Chem., 29, 468 (1986); Jones et al., Quinazoline Antifolates Inhibiting Thymidylate Synthase: Variation of the Amino Acid, J. Med. Chem., 29, 1114 (1986); and Jones et al., Quinazoline Antifolates Inhibiting Thymidylate Synthase: Variation of the N^{10} Substituent, J. Med. Chem. 28, 1468 (1985); and copending U.S. Patent Application Serial No. 07/432,338 filed November 6, 1989.

SUMMARY OF THE INVENTION

The present invention introduces a novel class of substituted tricyclic compounds which do not particularly resemble the structure of folic acid and yet, unexpectedly, inhibit the enzyme TS. The present invention also relates to pharmaceutical compositions containing these substituted tricyclic compounds and the use of these compounds to inhibit TS, including all effects derived from the inhibition of TS. Effects derived from the inhibition of TS include the inhibition of the growth and proliferation of the cells of higher organisms and of microorganisms, such as yeast and fungi. Such effects include antitumor activity. Processes for the preparation of the substituted tricyclic compounds of the invention are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to antiproliferative tricyclic compounds capable of inhibiting thymidylate synthase having the Formula (Q):



(Q)

wherein:

X and Y form a five- or six-membered heterocyclic ring containing at least one nitrogen;

Z is a hydrogen, halogen, carbon, oxygen or nitrogen atom;

U is a carbon or nitrogen atom;

n is 0 or the integer 1;

V is a carbon or nitrogen atom;

W is a carbon or nitrogen atom;

A is in the 1- or 2-position and is a nitrogen atom, a sulfur atom, or a substituted or unsubstituted alkylene group;

Ar is an aryl or heteroaryl group having one or more rings; and

B is either (i) an oxygen or nitrogen atom, or a $-CO-$ or $-SO_2-$ group, any of which is linked to an amino acid, aryl group, heterocyclic group or alkyl group, or (ii) a substituted or unsubstituted alkyl group.

As used herein, the expression "a compound capable of inhibiting thymidylate synthase" denotes a compound with a TS inhibition constant K_i of less than or equal to about 10^{-4}M. The compounds of the invention preferably have K_i values in the range of less than about 10^{-5}M, preferably less than about 10^{-6}M, even more

preferably less than about 10^{-9}M and, most preferably, in the range from about 10^{-12} to about 10^{-14}M.

X and Y in Formula (Q) can form any five- or six-membered heterocyclic ring containing at least one nitrogen such as, for example, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, and pyridazine rings. Preferably, X and Y form the ring:

```
        N
   \  /  \\
    C     \\
    ‖      C--P
    C      /
   /  \   /
       NH
```

wherein P is hydrogen; a lower alkyl group such as methyl, ethyl, propyl, isopropyl, tert-butyl and the like; or an amino group $-NR^1R^2$ wherein R^1 and R^2 independently represent hydrogen, alkyl, amino, hydroxyl, or the like.

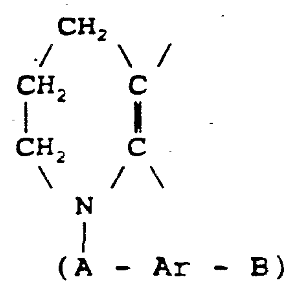
Z in Formula (Q) can be a hydrogen atom; a halogen atom such as chloro, bromo, or fluoro; a carbon atom which, taken with other appropriate atoms, may form such groups as substituted or unsubstituted alkyl, alkenyl, alkynyl, alkyl-oxy-alkylene, allyl, benzyl, acetyl, carbamyl, carbalkoxy, cyano, phenylacetyl, aminoalkyl or the like; an oxygen atom which, taken together with other appropriate atoms, may form such groups as hydroxy, alkoxy, oxamido, oxamyl, acetoxy, phenoxy, phenylsulfamyl, phenylsulfonamido or the like; or a nitrogen atom which, taken with other appropriate atoms, may form such groups as amino, nitro, acetamido, anilino, benzamido, formamido, hydrazino, hydroxamino, isocyano, nitramino, nitroso, oxamido, sulfamido, alkylamino, or the like. Preferably, z is a hydrogen atom.

The integer n in Formula (Q) can be 0 or 1, but is preferably 0. In other words, the left-hand ring in

the formula as written above is either a 6- or 7-membered ring.

U, V and W in Formula (Q) are each independently carbon or nitrogen atoms and, taken together with $(CH_2)_n$ and other appropriate carbon atoms as indicated in the formula, form a 6- or 7-membered ring such as, for example, a benzene, cyclohexene, pyridine, tetrahydropyridine, pyridazine, pyrimidine, 1,2,3-triazine, cycloheptene, tetrahydroazepine or the like ring.

In a preferred embodiment, U is a carbon atom, V is a carbon atom, and W is a nitrogen atom. Most preferably, U, V and W are, taken together with $(CH_2)_n$ and one or more other carbon atoms as indicated in the above formula, form a ring having the following structure:



Generally, A in Formula (Q) above is in the 1- or 2-position of the ring formed by U, $-(CH_2)_n$, V, W and the other appropriate atoms referred to above. A can be a nitrogen atom which, taken with other appropriate atoms, may form such groups as di- or trisubstituted amine or the like; a sulfur atom which, taken with other appropriate atoms, may form such groups as a thio linkage (-S-), thioalkylene, thioamide and the like; or any substituted or unsubstituted alkylene group such as, for example, methylene, ethylene, n-propylene, isopropylene, n-butylene, tert-butylene, n-hexylene or the like. It should be noted that, if A is sulfur, then W and V should be carbon to produce a reasonably stable compound. Preferably, A is a substituted or

unsubstituted alkylene group such as, for example, methylene, ethylene, n-propylene, isopropylene, n-butylene, tert-butylene, n-hexylene, or the like.

As indicated above, Ar can be any one of a large number of aryl or heteroaryl groups having one or more rings. Examples of useful aryl ring groups include phenyl, 1,2,3,4-tetrahydronaphthyl, naphthyl, phenanthryl, anthryl and the like. Examples of typical heteroaryl groups include 5-membered monocyclic ring groups such as thienyl, pyrrolyl, 2H-pyrrolyl, imidazolyl, pyrazolyl, furyl, isothiazolyl, furazanyl, isoxazolyl and the like; 6-membered monocyclic groups such as pyridyl, pyranyl, pyrazinyl, pyrimidinyl, pyridazinyl and the like; and polycyclic heteroaryl groups such as benzo[b]thienyl, naphtho[2,3-b]thienyl, thianthrenyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathiinyl, indolizinyl, isoindolyl, 3H-indolyl, indolyl, indazolyl, purinyl, 4H-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4H-carbazolyl, carbazolyl, beta-carbolinyl, phenanthridinyl, acridinyl, perimidinyl, phenathrolinyl, phenazinyl, isothiazlyl, phenothiazinyl, phenoxazinyl and the like. Preferably, Ar is a monocyclic or bicyclic aryl group, such as phenyl or naphthyl.

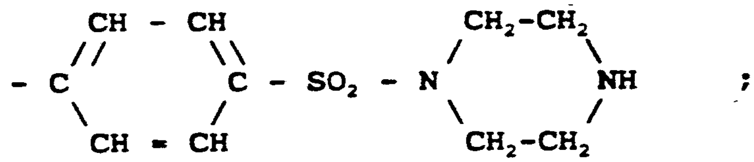
B, a substituent on the Ar group discussed above, can be an oxygen atom which may be taken alone to form an ether linkage (-O-) or which may be taken together with other appropriate atoms to form such groups as hydroxy, alkylenoxy, oxamido, oxamyl, acetoxy, phenoxy, phenylsulfamyl, phenylsulfonamido or the like; or a nitrogen atom which, taken with other appropriate atoms, may form such groups as amino NR^1R^2 wherein R^1 and R^2 can each independently be alkyl, or the like, nitro, acetamido, anilino, benzamido, formamido, hydrazino, hydroxamino, isocyano, nitramino, nitroso,

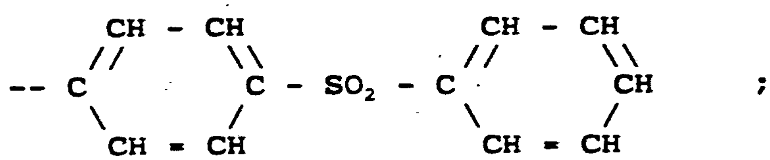
oxamido, sulfamido or the like; or a -CO- or $-SO_2-$ group.

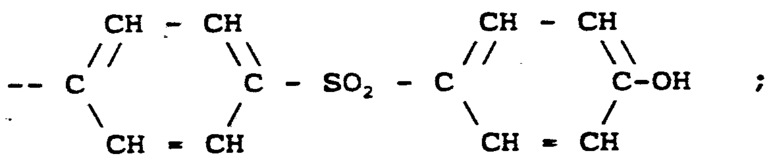
Any one of the divalent B groups above may be further linked to an amino acid group such as alanine, arginine, aspargine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine or the like; an aryl group such as phenyl, naphthyl and the like; a heterocyclic group such as thienyl, pyrrolyl, imidazolyl, pyrazolyl, isothiazolyl, pyrimidinyl, pyrazinyl, tetrahydroyrazinyl, benzo[b]thienyl, naphtho[2,3-b]thienyl, phoxathiinyl, indazolyl, phthalazinyl, cinnolinyl, carbolinyl, phenanthrolinyl, phenoxazinyl, and the like; or an alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, tert-butyl, n-hexyl and the like. Finally, B itself may be an alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, tert-butyl, n-hexyl or the like.

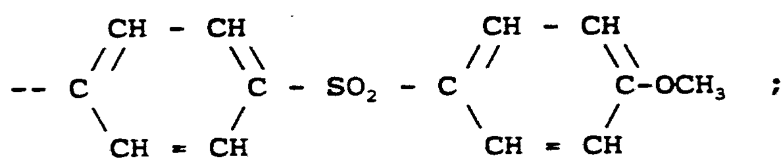
In a preferred embodiment, B is an -CO- or $-SO_2-$ group linked to an aryl or heterocyclic group, preferably phenyl or tetrahydroyrazinyl. When B includes an aryl group, the aryl group may be unsubstituted or may be substituted with one or more of a wide variety of electron-donating and electron-withdrawing substituents. Typical substituents include halogen, hydroxy, alkoxy, alkyl, hydroxyalkyl, fluoroalkyl, amino, -CN, $-NO_2$, carbalkoxy, carbamyl, carbonyl, carboxyldioxy, carboxy, amino acid carbonyl, amino acid sulfonyl, sulfamyl, sulfanilyl, sulfhydryl, sulfino, sulfinyl, sulfo, sulfonamido, sulfonyl or the like. Most preferably, B is an $-SO_2-$ group linked to a phenyl group which is either unsubstituted or substituted in the para-position with a hydroxy group or an alkoxy group such as methoxy, ethoxy, iso-propoxy, tert-butoxy, chloroethoxy or the like.

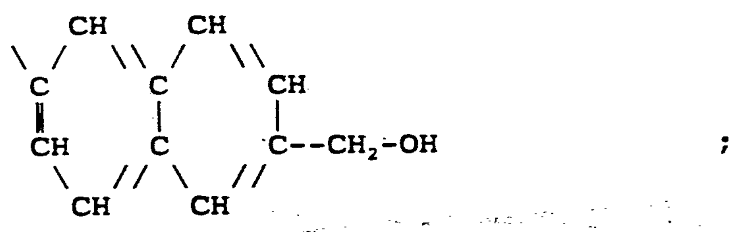
Particularly preferred structures for -Ar-B include:

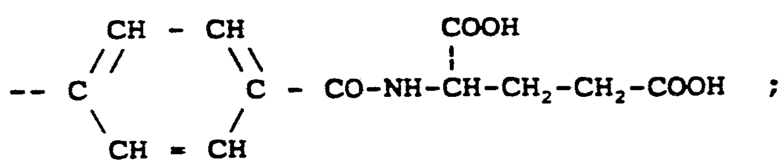


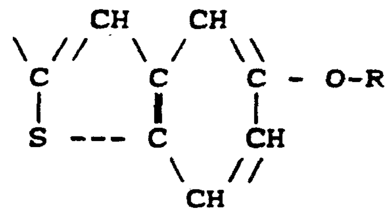




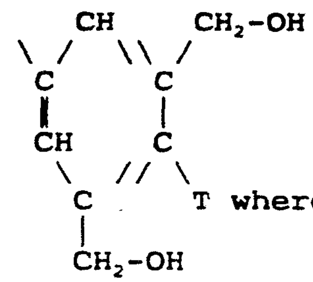








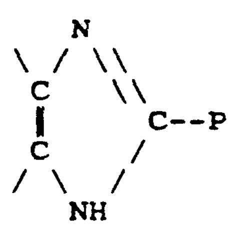
wherein R is H or alkyl, such as methyl; and



wherein T is H or -CN.

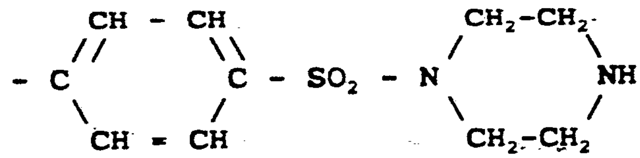
In addition to the substituent B defined above, Ar in Formula (Q) can also be substituted with one or more of a wide variety of electron-donating and electron-withdrawing substituents. Typical substituents include halogen, hydroxy, alkoxy, alkyl, hydroxyalkyl, fluoroalkyl, amino, -CN, $-NO_2$, carbalkoxy, carbamyl, carbonyl, carboxyldioxy, carboxy, amino acid carbonyl, amino acid sulfonyl, sulfamyl, sulfanilyl, sulfhydryl, sulfino, sulfinyl, sulfo, sulfonamido, sulfonyl or the like. Preferably, these additional substituents are selected from the group consisting of -CN, fluoroalkyl, and sulfonyl.

In a particularly preferred group of compounds capable of inhibiting thymidylate synthase according to the present invention, X and Y in Formula (Q) form the ring:



wherein P is lower alkyl such as methyl, amino or hydrogen; U is carbon; n is 0; V is carbon; W is

nitrogen; A is methylene; and -Ar-B is selected from the group consisting of:



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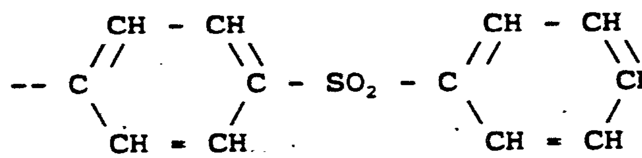

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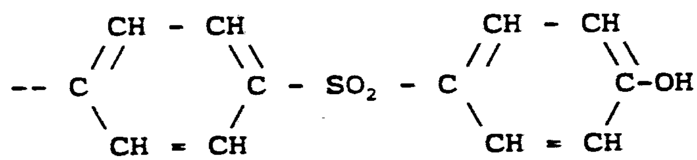

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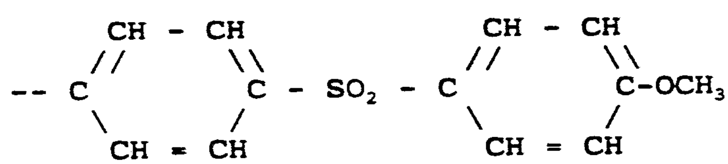

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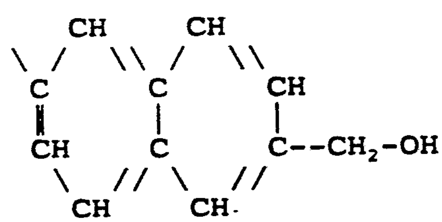

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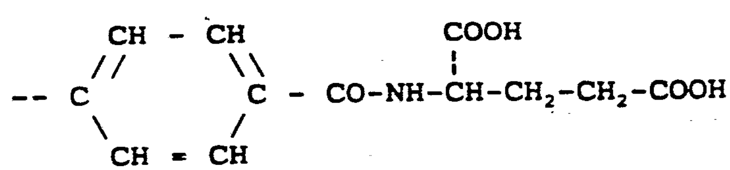

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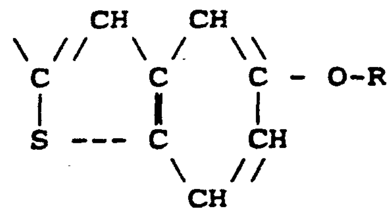
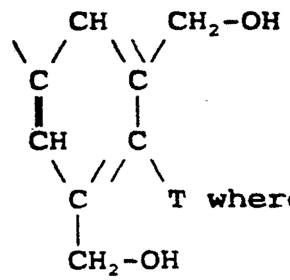
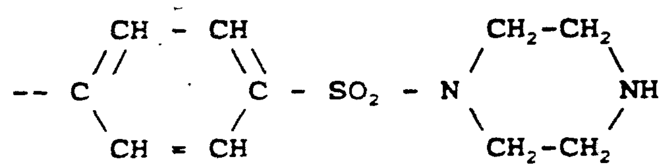


wherein R is H or alkyl, such as methyl; and



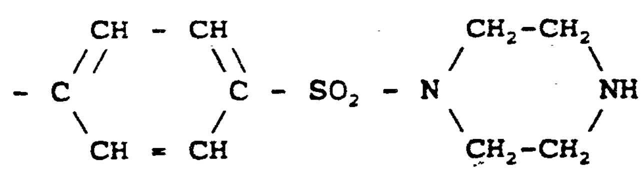
wherein T is H or -CN.

Examples of particularly preferred compounds of the invention include those falling within the above group wherein P is hydrogen and -Ar-B is



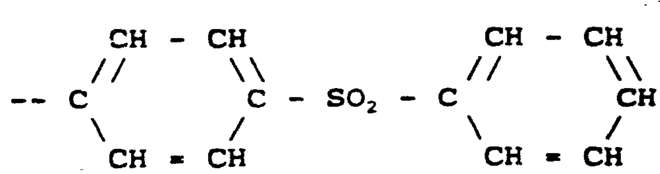
;

wherein P is $-NH_2$ and -Ar-B is



;

wherein P is $-NH_2$ and -Ar-B is



;

wherein P is $-NH_2$ and -Ar-B is

```
     CH      CH
  \ /  \\  /   \\
   C      C      CH
   ‖      |      |
   CH     C      C--CH2-OH                 ;
    \   //  \   //
     CH      CH
```

wherein P is $-NH_2$ and -Ar-B is

```
      CH - CH                  CH - CH
     //      \\               //      \\
 -- C          C - SO2 - C          C-OH    ;
     \        /               \        /
      CH = CH                  CH = CH
```

wherein P is $-CH_3$ and -Ar-B is

```
      CH - CH                  CH - CH
     //      \\               //      \\
 -- C          C - SO2 - C          C-OCH3   ;
     \        /               \        /
      CH = CH                  CH = CH
```

wherein P is $-NH_2$ and -Ar-B is

```
      CH - CH                  CH - CH
     //      \\               //      \\
 -- C          C - SO2 - C          C-OCH3   ;
     \        /               \        /
      CH = CH                  CH = CH
```

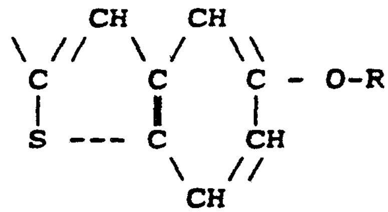
wherein P is $-NHCH_3$ and -Ar-B is

```
      CH - CH                  CH - CH
     //      \\               //      \\
 -- C          C - SO2 - C          C-OCH3   ;
     \        /               \        /
      CH = CH                  CH = CH
```

wherein P is $-NH_2$ and -Ar-B is

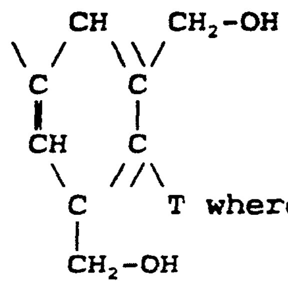
```
      CH - CH              COOH
     //      \\             |
 -- C          C - CO-NH-CH-CH2-CH2-COOH   ;
     \        /
      CH = CH
```

wherein P is $-NH_2$ and -Ar-B is



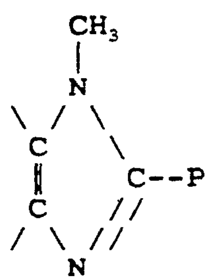
wherein R is H or alkyl, such as methyl; and

wherein P is $-NH_2$ and -Ar-B is



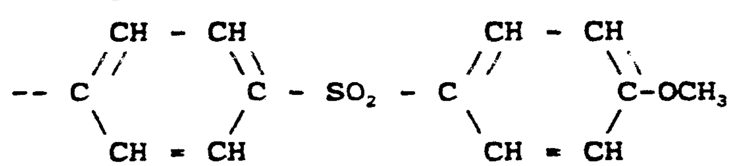
wherein T is H or -CN.

In another particularly preferred embodiment according to the present invention, X and Y in Formula (Q) form the ring:



;

wherein P is selected from the group consisting of $-NH_2$ and methyl; U is carbon; n is 0; V is carbon; W is nitrogen; A is methylene; and -Ar-B is



.

In a still further preferred group of compounds according to the present invention, X and Y in Formula (Q) form the ring:

```
          N
       \ / \\
        C    \\
        ‖     C--P
        C    /
       / \  /
          NH
```

wherein P is selected from the group consisting of -NHOH and $-NHNH_2$; U is carbon; n is 0; V is carbon; W is nitrogen; A is methylene; and -Ar-B is

```
        CH - CH                CH - CH
       //      \\             //      \\
   -- C         C - SO2 - C          C-OCH3    .
       \       /             \       /
        CH = CH                CH = CH
```

In a still further particularly preferred compound capable of inhibiting thymidylate synthase according to the present invention, X and Y in Formula (Q) form the ring:

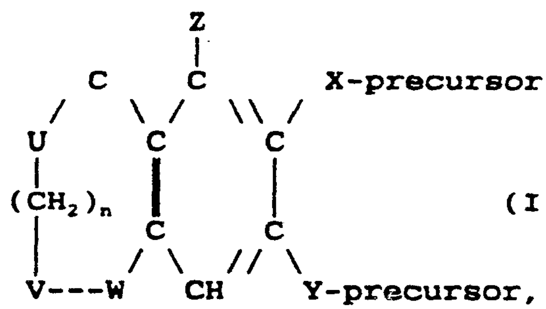
```
          N
       \ / \\
        C    \\
        ‖     C--NH2        ;
        C    /
       / \  /
          NH
```

U is carbon; n is 0; V is carbon; W is carbon; A is divalent sulfur; and -Ar-B is

```
        CH - CH                CH - CH
       //      \\             //      \\
   -- C         C - SO2 - C          CH    .
       \       /             \       /
        CH = CH                CH = CH
```

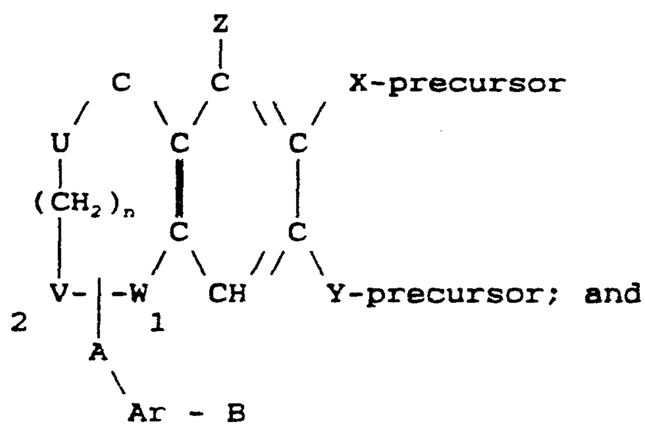
The invention also relates to a process for making the compounds of the present invention comprising the steps of:

(1) allowing a compound of the formula B-Ar-A-D, wherein A, Ar and B are as defined above and D is a displaceable group, to react with a compound of Formula (I):



(I)

wherein X-precursor and Y-precursor are groups which, when cyclized with each other, form a five- or six-membered heterocyclic ring containing at least one nitrogen, to form a substituted compound having the formula:



(2) cyclizing X-precursor and Y-precursor to form with each other a five- or six-membered heterocyclic ring containing at least one nitrogen atom.

The displaceable group D can be any group which is displaceable under the reaction conditions used and is typically a halogeno such as fluoro, chloro or bromo; a substituted sulfonyloxy such as methanesulfonyloxy, trifluoromethanesulfonyloxy, toluene-p-sulfonyloxy, or 4-bromobenzenesulfonyloxy; an aldehyde; or the like. A halogeno is a preferred displaceable group, with bromo being particularly preferred.

The first step, reacting B-Br-A-D with a compound of Formula (I), may be carried out in an organic

solvent. Typically, when an organic solvent is used, it is an aprotic solvent such as dimethylformamide, dimethylacetamide, dimethylsulfoxide, or tetrahydrofuran. Especially preferred solvents include dimethylformamide and dimethylacetamide.

Typically, also, the first step is carried out in the presence of a weak base which will not itself react with one of the reactants. Useful bases include, for example, organic bases such as a substituted amine such as diisopropylethylamine, dimethyl-sec-butylamine, N-methyl-N-ethylaniline, N,N-dimethylaniline or the like; and inorganic weak bases such as sodium, potassium and/or calcium carbonate or the like. The reaction temperature for the first step can vary from about room temperature to about 100°C, but preferably ranges from about 65°C to about 85°C.

The second step of cyclizing X-precursor and Y-precursor with each other to form a five- or six-membered heterocyclic ring containing at least one nitrogen atom is carried out in the presence of a cyclizing agent such as $HC(OCH_3)_3/HCl$, CNBr, $CH_3C(OCH_3/HCl$ or a combination of a metal such as tin and acetic acid. Most preferably, the cyclizing agent is $HC(OCH_3)_3/HCl$ or CNBr.

The reaction may be carried out in the presence of an organic solvent, such as methanol, ethanol, butanol, acetonitrile, mixtures thereof or the like. When the cyclizing agent is CNBr, for example, a mixture of methanol and acetonitrile can advantageously be used. In contrast, however, when the cyclizing agent is $HC(OCH_3)_3/HCl$, the starting material is typically dissolved in the $HC(OCH_3)_3$ itself without any additional solvent, and a relatively small amount of HCl is then added to initiate the cyclizing step.

The temperature used for the cyclizing step ranges from just below room temperature to about 70°C, but is preferably from about 20 to about 60°C.

It should be noted that one or more of X-precursor, Y-precursor, U, V and W may contain a chemical group or groups which, either before, after or during the course of either the substitution step (1) or the cyclizing step (2):

(a) may be protected by a protecting group or

(b) may have one or more of any protecting groups present removed.

A suitable protecting group for a ring nitrogen, such as U, V or W may be, for example, a pivaloyloxymethyl group, which may be removed by hydrolysis with a base such as sodium hydroxide; a tert-butyloxycarbonyl group, which may be removed by hydrolysis with an acid such as hydrochloric acid or with a base such as lithium hydroxide; or a 2-(trimethylsilyl)ethoxymethyl group, which may be removed by a fluoride salt such as tetra-n-butyl ammonium flouride or with an acid such as hydrochloric acid.

A suitable protecting group for a hydroxyl group is, for example, an esterifying group such as an acetyl or benzoyl group, which may be removed by hydrolysis with a base such as sodium hydroxide. Alternatively, when other groups present in the starting material do not contain an alkenyl or alkynyl group, the protecting group may be, for example, an alpha-arylalkyl group such as a benzyl group, which may be removed by hydrogenation in the presence of a catalyst such as palladium-on-charcoal or Raney nickel.

A suitable protective group for a mercapto group is, for example, an esterifying group such as an acetyl group, which may be removed by hydrolysis with a base such as sodium hydroxide.

A suitable protective group for an amino group may be, for example, an alkylcarbonyl group such as an acetyl group (CH_3CO-) or a benzoyl group, which may be removed by treatment with an inorganic acid such as

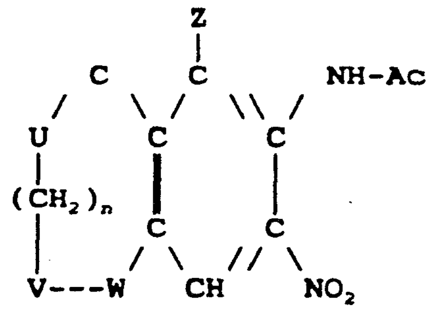
nitric, sulfuric or hydrochloric acid, or by base hydrolysis with sodium hydroxide. Another protective group for an amino group is an alkoxycarbonyl group such as a methoxycarbonyl or a tert-butyloxycarbonyl group. These groups may be removed by treatment with an organic acid such as trifluoroacetic acid. Alternatively, the protective group may be a benzyloxycarbonyl group, which may be removed by treatment with a Lewis acid such as boron tris(trifluoroacetate) or hydrogen gas in the presence of a palladium-on-charcoal catalyst.

A suitable protective group for a primary amino group is, for example, an alkylcarbonyl group such as acetyl, which may be removed by treatment with an inorganic acid such as nitric, sulfuric or hydrochloric acid, or a phthaloyl group, which may be removed by treatment with an alkylamine such as 3-dimethylaminopropyl amine, with hydrazine, or with ammonia.

A suitable protective group for a carboxy group may be an esterifying group, for example, a methyl or an ethyl group, which may be removed by hydrolysis with a base such as sodium hydroxide. Another useful protecting group is a tert-butyl group, which may be removed by treatment with an organic acid such as trifluoroacetic acid.

Preferred protective groups include an esterifying group, an alpha-arylalkyl group, an alkylcarbonyl group, a substituted or unsubstituted alkoxycarbonyl group, a phthaloyl group, a pivaloyloxymethyl group or a methyl oxyether-type group such as methoxymethyl or 2-(trimethylsilyl)ethoxymethyl.

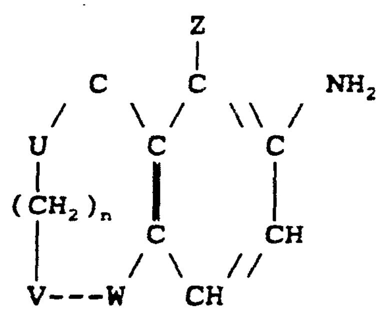
A particular aspect of the invention relates to a process of making a substituted tricyclic compound capable of inhibiting thymidylate synthase from a starting compound of Formula (I), wherein the compound of Formula (I) has the structure of Formula (II):



(II)

wherein Ac is a CH_3CO- protective group. A process of making this group of starting compounds may comprise the steps of:

(1) selectively protecting an amine compound of Formula III:



(III)

to form the corresponding acetamide; and

(2) nitrating the corresponding acetamide to form the compound of Formula (II).

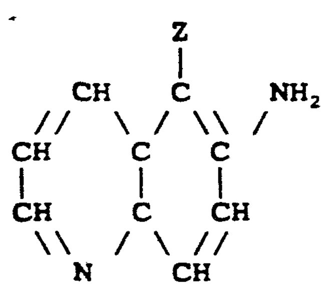
In the first step, adding a protective group to a compound of Formula II to form the corresponding acetamide is preferably performed by treating the amine compound of Formula (III) with an appropriate anhydride compound, such as acetic anhydride, in the presence of an organic solvent, such as pyridine or the like. The addition of a protective group can occur at temperatures lower or higher than room temperature. Typically, however, the addition of a protective group occurs at a temperature between about -10 and +15°C and, most preferably, between about -10 and -5°C.

The second step of nitrating the acetamide formed by selectively protecting the amine compound can be carried out in the presence of one or more of the many

known nitrating agents, such as (1) a mixture of nitric and sulfuric acids; (2) a mixture of nitric, sulfuric and acetic acids; or (3) a mixture of nitric acid and acetic anhydride. Preferably, the nitrating agent is a mixture of nitric and sulfuric acids. The nitration step may be carried out over a wide range of temperatures but is typically carried out at a temperature between about -10 and +10°C, preferably between about -10 and 5°C.

The amine compound of Formula (III) itself can be prepared by several different reaction schemes. In one embodiment, the amine compound of Formula (III) is prepared by reducing a compound corresponding to the amine having one or more sites of unsaturation in the 6- or 7-membered ring formed by U, V and W taken in combination with an appropriate number of carbon and hydrogen atoms. In this embodiment, the reduction can be performed under widely varying reduction conditions, but is preferably carried out in water or in an organic solvent such as methanol, ethanol, tetrahydrofuran, acetic acid or the like, in the presence of a reducing agent such as a hydrazine compound or hydrogen gas at a pressure of at least one atmosphere, preferably at a pressure from about 1 to about 50 psi. A reduction catalyst is also used, such as platinum oxide (as described in Ishikawa et al., Chem. Pharm. Bull., 37, 2103 (1989) which is hereby incorporated by reference).

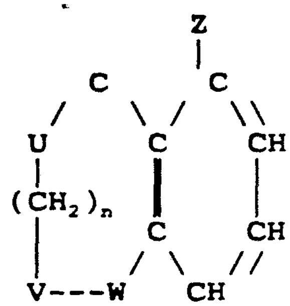
In a most preferred embodiment, the compound corresponding to the amine having one or more sites of unsaturation has the formula:



and is reduced by treatment with hydrogen gas and a platinum oxide catalyst in an organic solvent such as glacial acetic acid.

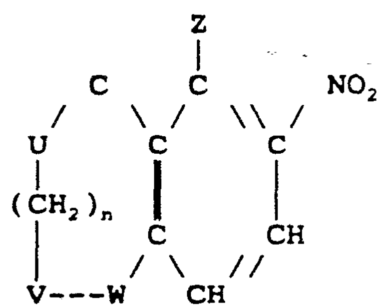
Alternatively, in another preferred embodiment, the amine compound of Formula (III) is prepared by:

(1) nitrating an unsubstituted compound of Formula (IV):



(IV)

to form a nitrated compound having the formula:



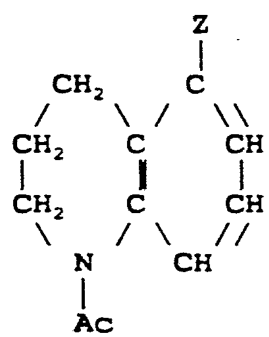
; and

(2) reducing the nitrated compound to form the amine of Formula (III).

The reaction conditions for the nitration step (1) are typically as described above for general nitration reactions. Preferably, however, the nitration step is performed in the presence of nitric acid and at a temperature between about -10 to about 20°C. A particularly preferred nitration process using these conditions is described in Amit et al., J. Chem. Soc. Perkins II, 57 (1976), which is hereby incorporated by reference.

The reaction conditions used for the reduction step (2) can vary greatly but, typically, include one or more of the following: (a) treatment with $SnCl_2$ in the presence of hydrochloric acid; (b) treatment with zinc in the presence of acetic acid; (c) treatment with $Fe^{+3}(CO)_{12}$ in benzene and methanol; (d) treatment with hydrogen gas in the presence of a palladium-on-charcoal catalyst; (e) treatment with hydrogen gas in the presence of a platinum oxide catalyst in an organic solvent such as glacial acetic acid; and (f) treatment with a hydrazine in the presence of a reduction catalyst. Preferably, however, the reduction step (2) is carried out with hydrogen gas as the reducing agent in the presence of a palladium-on-charcoal catalyst in an organic solvent, for example, an alcohol such as methanol.

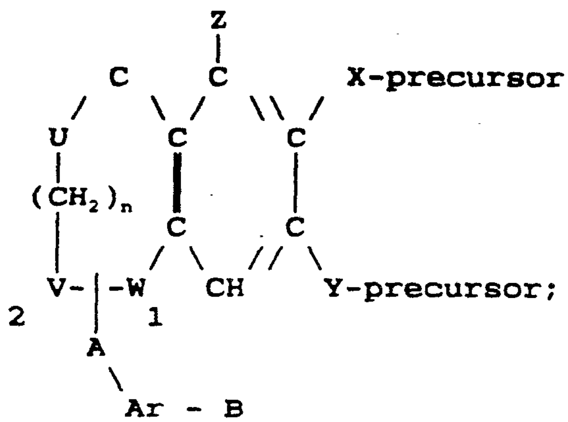
Most preferably, the compound of Formula (IV) has the formula:



(IV)

wherein Ac is an acetyl (CH_3CO-) protective group which is removed between the nitration step and the reduction step. The removal of this protective group can be accomplished by the general methods described above for removal of protective groups from amino groups. Preferably, the acetyl group is removed by treatment with an inorganic acid such as hydrochloric acid in a solvent, for example, water, an alcohol such as ethanol, or a mixture of water and an alcohol at a temperature about the boiling point of the solvent.

As stated above, X-precursor and Y-precursor are groups which, when cyclized with each other, form a five- or six-membered heterocyclic ring containing at least one nitrogen. In a preferred embodiment, in the compound



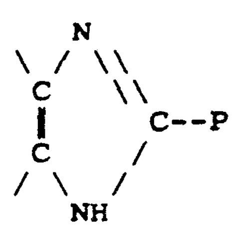
X-precursor is -NH-Ac and Y-precursor is $-NO_2$. In this embodiment, the -NH-Ac and $-NO_2$ groups can be cyclized with each other using one of several alternative methods, two of which are described below as methods (A) and (B).

In method (A), the compound is treated successively with:

(1) a deprotecting agent, for example, an inorganic acid such as hydrochloric acid, to convert the -NH-Ac group to a free amino group;

(2) a reducing agent, for example, hydrazine in the presence of a reduction catalyst such as Raney nickel; and

(3) a cyclizing agent, such as $HC(OCH_3)_3$, $CH_3C(OCH_3)_3$ or CNBr, to form a ring having the formula:



wherein P is an alkyl group such as a methyl group, an amino group or hydrogen. In method (A), the cyclizing agent selected will determine the identity of P. For example, if the cyclizing agent selected is $HC(OCH_3)_3$, P will be hydrogen; if the cyclizing agent is $CH_3C(OCH_3)_3$, P will be a methyl group, and if the cyclizing agent is CNBr, P will be the amino.

In an alternative method (B), the compound containing an -NH-Ac group as X-precursor and an $-NO_2$ group as Y-precursor is treated directly with a metal such as tin, zinc, or the like, in the presence of acetic acid. P will then be methyl in the resulting cyclized compound. While either method (A) or method (B) is useful, as can be a variety of other known cyclizing methods, cyclizing method (B) is preferred.

Another aspect of the invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier in combination with at least one compound according to the present invention in an amount effective to inhibit thymidylate synthase. The composition preferably contains a total amount of a compound of the invention which is an efficacious amount.

The substituted tricyclic compounds of the present invention which may be employed in the pharmaceutical compositions of the invention include all of those compounds described above, as well as the pharmaceutically acceptable salts of these compounds. Pharmaceutically acceptable acid addition salts of the compounds of the invention containing a basic group are formed where appropriate with strong or moderately strong organic or inorganic acids in the presence of a basic amine by methods known to the art. Exemplary of the acid addition salts which are included in this invention are maleate, fumarate, lactate, oxalate, methanesulfonate, ethanesulfonate, benzenesulfonate,

tartrate, citrate, hydrochloride, hydrobromide, sulfate, phosphate and nitrate salts. Pharmaceutically acceptable base addition salts of compounds of the invention containing an acidic group are prepared by known methods from organic and inorganic bases and include, for example, nontoxic alkali metal and alkaline earth bases, such as calcium, sodium, potassium and ammonium hydroxide; and nontoxic organic bases such as triethylamine, butylamine, piperazine, and tri(hydroxymethyl)methylamine.

As stated above, the compounds of the invention possess antiproliferative activity, a property which may express itself in the form of antitumor activity. A compound of the invention may be active per se or it may be a pro-drug that is converted in vivo to an active compound. Preferred compounds of the invention are active in inhibiting the growth of the L1210 cell line, a mouse leukemia cell line which can be grown in tissue culture. Such compounds of the invention are also active in inhibiting the growth of bacteria such as Escherichia coli, a gram-negative bacterium which can be grown in culture.

The substituted tricyclic compounds according to the present invention, as well as the pharmaceutically acceptable salts thereof, may be incorporated into convenient dosage forms such as capsules, tablets or injectable preparations. Solid or liquid pharmaceutically acceptable carriers may be employed. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline and water. Similarly, the carrier or diluent may include any prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir,

emulsion, soft gelatin capsule, sterile injectable liquid (e.g. solution), such as an ampoule, or an aqueous or nonaqueous liquid suspension.

The pharmaceutical preparations are made following conventional techniques of a pharmaceutical chemist involving steps such as mixing, granulating and compressing, when necessary, for tablet forms, or mixing, filling and dissolving the ingredients, as appropriate, to give the desired products for oral, parenteral, topical, intravaginal, intranasal, intrabronchial, intraocular, intraaural and rectal administration.

The compositions of the invention may further comprise one or more other compounds which are antitumor agents, such as mitotic inhibitors (e.g., vinblastine), alkylating agents (e.g., cis-platin, carboplatin and cyclophosphamide), DHFR inhibitors (e.g., methotrexate, piritrexim or trimetrexate), antimetabolites (e.g., 5-fluorouracil and cytosine arabinoside), intercalating antibiotics (e.g., adriamycin and bleomycin), enzymes (e.g., asparaginase), topoisomerase inhibitors (e.g., etoposide) or biological response modifiers (e.g., interferon).

The composition of the invention may also comprise one or more other compounds including antibacterial, antifungal, antiparasitic, antiviral, antipsoriatic and anticoccidial agents. Exemplary antibacterial agents include, for example, sulfonamides such as sulfamethoxazole, sulfadiazine, sulfameter or sulfadoxine; DHFR inhibitors such as trimethoprim, bromodiaprim or trimetrexate; penicillins; cephalosporins; aminoglycosides; bacteriostatic inhibitors of protein synthesis; the quinolonecarboxylic acids and their fused isothiazolo analogs.

Another aspect of the invention relates to a therapeutic process of inhibiting thymidylate synthase, which process comprises administering to a vertebrate host such as a mammal or bird an amount effective to inhibit thymidylate synthase of a tricyclic compound according to the present invention. The compounds of the invention are particularly useful in the treatment of mammalian hosts, such as human hosts, and in the treatment of avian hosts.

Any of the substituted tricyclic compounds described above, or pharmaceutically acceptable salts thereof, may be employed in the therapeutic process of the invention. The compounds of the invention may be administered in the therapeutic process of the invention in the form of a pharmaceutically acceptable composition comprising a diluent or carrier, such as those described above. Doses of the compounds preferably include pharmaceutical dosage units comprising an efficacious quantity of active compound. By an efficacious quantity is meant a quantity sufficient to inhibit TS and derive the beneficial effects therefrom through administration of one or more of the pharmaceutical dosage units. An exemplary daily dosage unit for a vertebrate host comprises an amount of up to about 5,000 mg of active compound per square meter of the body area of the vertebrate host.

The selected dose may be administered to a warmblooded animal or mammal, for example a human patient, in need of treatment mediated by thymidylate synthase inhibition by any known method of administration, including topically (e.g., as an ointment or cream), orally, rectally (e.g., as a suppository), parentally, by injection or continuously by infusion, intravaginally, intranasally, intrabronchially, intra-aurally or intraocularly.

The substituted tricyclic compounds according to the present invention may be further characterized as

producing any one or more of an antiproliferative effect, an antibacterial effect, an antiparasitic effect, an antiviral effect, an antipsoriatic effect, an antiprotozoal effect, an anticoccidial effect or an antifungal effect. The compounds are especially useful in producing an antitumor effect in a vertebrate host harboring a tumor.

The compounds of the present invention are antagonists of a folate cofactor and therefore may affect one or more other folate-dependent enzymatic systems as well. Examples of other folate-dependent enzymatic systems which may be affected include 5,10-methylenetetrahydrofolate reductase, serine hydroxymethyltransferase, and glycineamineribotide transformylase.

The following examples illustrate the invention, although the scope and spirit of the invention are not limited thereto.

EXAMPLES

The structures of all compounds of the invention were confirmed by proton magnetic resonance spectroscopy, infrared spectroscopy, elemental microanalysis or, in certain cases, by mass spectrometry.

Proton magnetic resonance spectra were determined using a General Electric QE-300 spectrometer operating at a field strength of 300MHz. Chemical shifts are reported in parts per million (δ) and by setting the references such that, in $CDCl_3$, the $CHCl_3$ peak is at 7.26 ppm and, in D_6DMSO, the DMSO peak is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; brs, broad singlet; brd, broad doublet; br, broad signal; and m, multiplet.

Mass spectra were determined using a VG 7070E-HF high resolution mass spectrometer using the direct insertion method, an ionizing voltage of 70eV, and an ion source temperature of 200°C. Infrared absorption spectra were taken on a Perkin-Elmer 457 spectrometer. Elemental microanalysis gave results for the elements stated with ±0.4% of the theoretical values.

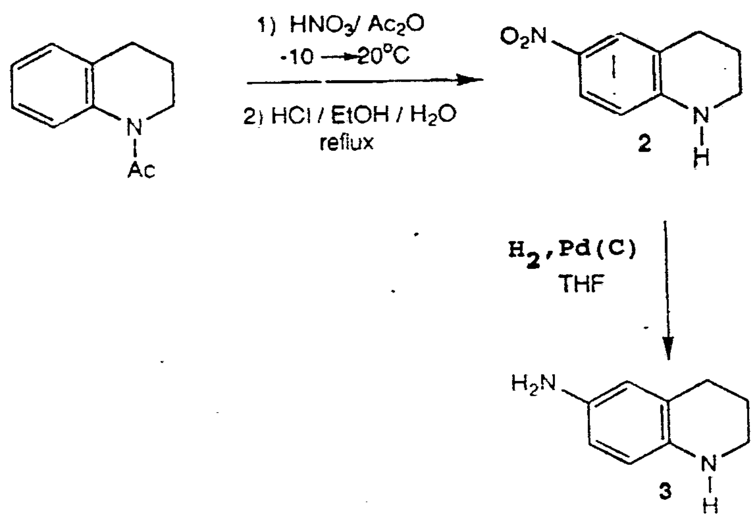
N-N-Dimethylformamide ("DMF") was dried over activated (250°) 3-Å molecular sieves, and N,N-dimethylacetamide ("DMA") (Aldrich Gold Label grade) was similarly dried. Tetrahydrofuran (THF") was distilled from sodium benzophenone ketyl under nitrogen. The term "ether" refers to diethyl ether. The term "petrol" refers to petroleum ether of b.p. 36-53°C.

Flash chromatography was performed using Silica gel 60 (Merck Art 9385). Where the crude solid was insoluble in the chosen eluant, it was dissolved in a more polar solvent, and Merck Art 7734 silica was added. The slurry was evaporated to dryness on a

rotary evaporator fitted with a course glass frit to prevent spraying of the silica. The coated silica was then applied to the column. Thin layer chromatographs ("TLC") were performed on precoated sheets of silica 60 F_{254} (Merck Art 5719). Extracts were dried over anhydrous Na_2SO_4 or $MgSO_4$. Melting points were determined on a Mel-Temp apparatus and are uncorrected.

<u>Example 1: Preparation of Compounds 2 and 3</u>

Compounds 2 and 3 were prepared according to the following reaction scheme:



Preparation of Compound 2 -
6-Nitrotetrahydroquinoline

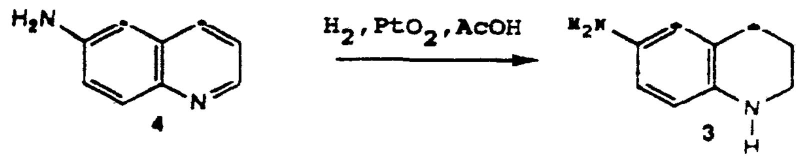
N-acyl tetrahydroquinoline, obtained by the acylation (acetic anhydride, pyridine) of tetrahydroquinoline, was nitrated and deprotected as described by Amit et al., J. Chem Soc. Perkins II, 57 (1976). ^{1}H NMR ($CDCl_3$) δ 7.86-7.90 (2H, m), 6.36 (1H, d, J=9.6Hz), 4.75 (1H, brs), 3.41 (2H, t, J=5.6Hz), 2.79 (2H, t, J=6.3Hz), and 1.91-1.99 (2H, m).

Preparation of Compound 3 -
6-Aminotetrahydroquinoline

6-Nitrotetrahydroquinoline, 2 (29.00g, 0.16 mol), in 200 ml MeOH and 10% palladium-on-charcoal (3.00g) was shaken on a Parr hydrogenator with 35 psi H_2 for 1.5

hours. The mixture was filtered through a diatomaceous earth material sold under the trade name Celite, concentrated, and purified by flash chromatography (50% - 75% EtOAc/Hexane) to give a yellow-brown solid, 15.79g (0.11 mol, 68%), m.p. 75-80°C. [1]H NMR ($CDCl_3$) δ 6.40 (3H, m), 3.23 (2H, t, J=5.4Hz), 2.69 (2H, t, J=6.5Hz), and 1.89-1.92 (2H, m). IR (KBr) 3400, 3360, 3900, 880, 810.

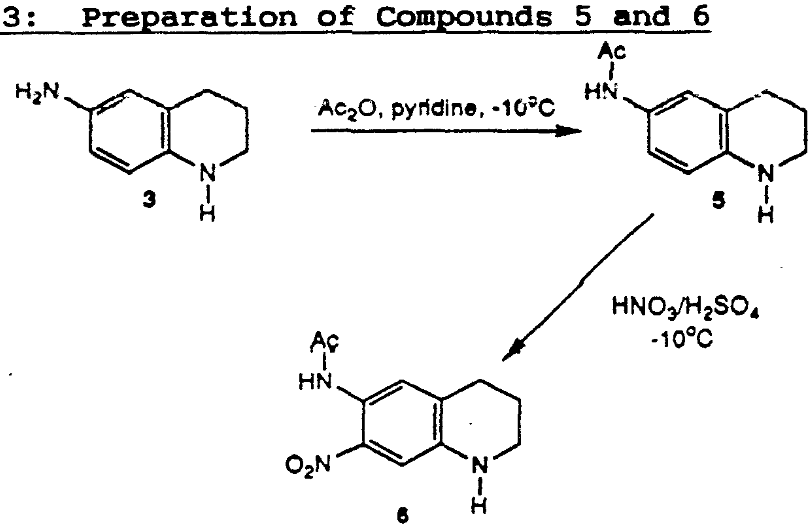
Example 2: Alternate Synthesis of Compound 3



6-Aminotetrahydroquinoline - Alternative Method

6-Aminoquinoline (1.00g, 6.93 mmol) in 20 ml glacial acetic acid over PtO_2 (0.06g, 0.30 mmol) was shaken at 45 psi for two hours in accordance with the procedure of Ishikawa et al., Chem. Pharm. Bull., 37, 2103 (1989). The mixture was filtered though a diatomaceous earth material sold under the trade name Celite, basified with 6N NaOH, and extracted with CH_2Cl_2 (2 x 100 ml). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated to a grey solid, 0.62g (4.18 mmol, 60%).

Example 3: Preparation of Compounds 5 and 6



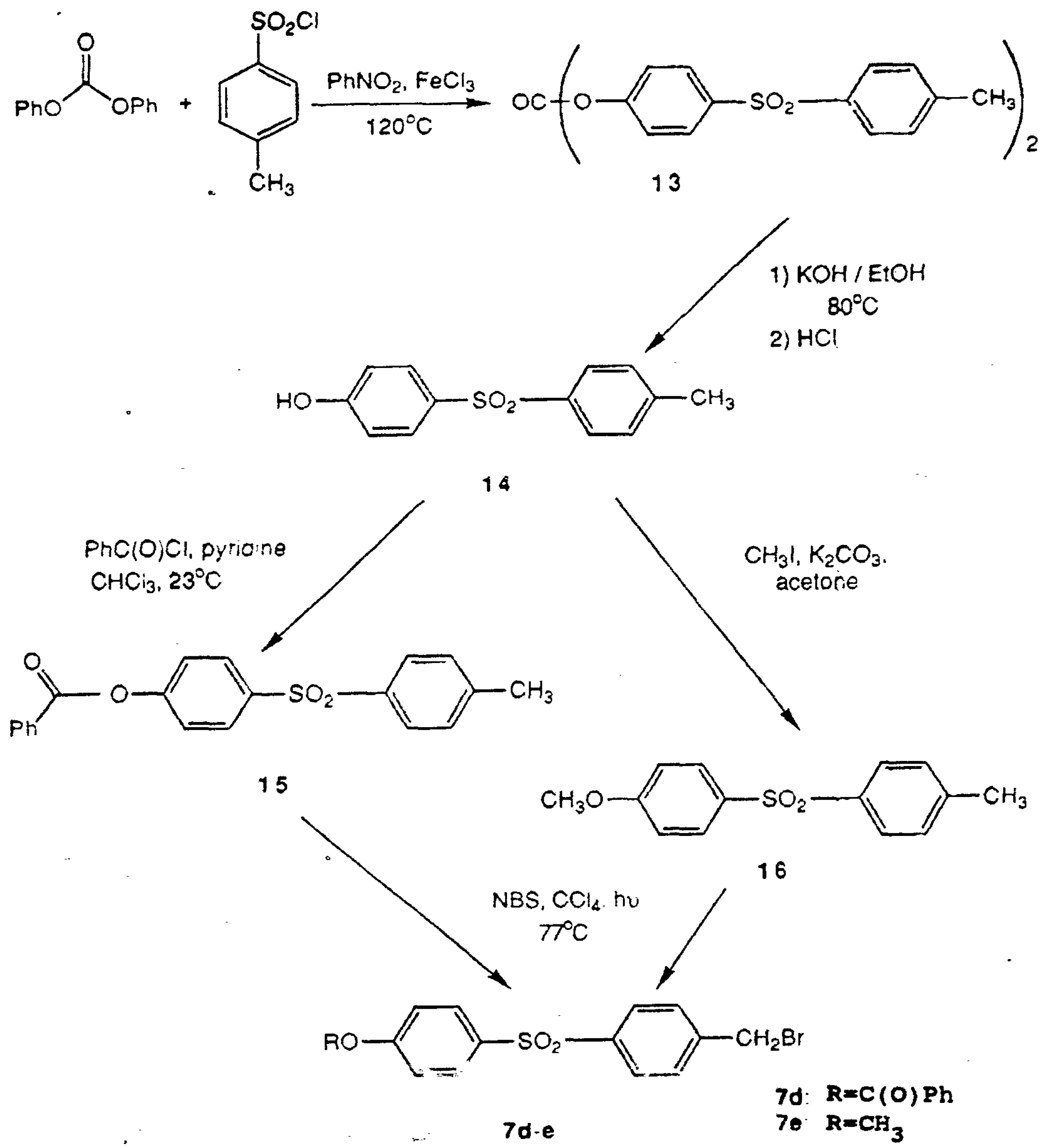
Preparation of Compound 6 -
6-Acylamino-7-nitrotetrahydroquinoline

The primary amino group of Compound 3 was selectively protected as the acetamide, Compound 5 (acetic anhydride, pyridine, -10°C).

A solution of Compound 5 (3.72g, 19.55 mmol) in 30 ml 98% H_2SO_4 at -10°C was treated with 70% HNO_3 (1.30 ml, 20.22 mmol), during which the temperature increased to 5°C. The mixture was poured into 400 ml water, neutralized with 6N NaOH, and extracted with ethyl acetate (3 x 500 ml). ^1H NMR of the crude material showed a 2.5:1 mixture of Compound 6 and its 5-nitro isomer. The extract was dried over anhydrous Na_2SO_4, concentrated, and purified by flash chromatography (65% EtOAc/Hexane) to give 2.58g of Compound 6 as a dark red solid (10.97 mmol, 56%), m.p. 150-155°C. ^1H NMR ($CDCl_3$) δ 9.80 (1H, brs), 8.22 (1H, s), 7.22 (1H, s), 4.10 (1H, brs), 3.32 (2H, t, J=5.5Hz), 2.82 (2H, t, J=6.4Hz), 2.22 (3H, s), and 1.91-1.95 (2H, m). IR (KBr) 3410, 3320, 2930, 1650, 1580, 850. HRMS, exact mass calculated for $C_{11}H_{13}N_3O_3$: M+ requires 235.0957. Found: 235.0950.

Example 4: Preparation of Compounds 13 through 16 and Compounds 7(d) and 7(e)

Compounds 13-16 and Compounds 7(d) and 7(e) were prepared according to the following reaction scheme:


SO2Cl
PhO OPh +
CH3
PhNO2, FeCl3
120°C
OC O SO2 CH3 2
13
1) KOH / EtOH
80°C
2) HCl
HO SO2 CH3
14
PhC(O)Cl, pyridine
CHCl3, 23°C
CH3I, K2CO3,
acetone
Ph O SO2 CH3
15
CH3O SO2 CH3
16
NBS, CCl4, hυ
77°C
RO SO2 CH2Br
7d-e
7d: R=C(O)Ph
7e: R=CH3

Preparation of Compound 13 -

Di-4-(p-toluenesulfonyl)phenyl carbonate

A solution of diphenyl carbonate (50.16g, 0.23 mol), p-toluene sulfonyl chloride (90.04g, 0.47 mol) and $FeCl_3$ (1.12g, 0.01 mol), in 75 ml nitrobenzene was heated to 120°C, while evolving HCl was bubbled through a water trap. After two hours, the mixture was cooled to 23°C, forming a light beige precipitate. The solid was filtered and washed with MeOH to give 87.61g crude Compound 13 (0.17 mol, 72%), m.p. 149-160°C. ^1H NMR ($CDCl_3$) δ 7.98 (2H, d, J=8.7Hz), 7.82 (2H, d, J=8.3Hz), 7.40 (2H, m), 7.24-7.32 (2H, m), and 2.40 (3H, s).

Preparation of Compound 14

4-(p-Toluenesulfonyl)phenol

A solution of Compound 13 (87.61g, 0.17 mol) in 150 EtOH/150 ml 5N KOH solution was heated to 80°C for one hour. The mixture was poured into 500 ml MeOH and neutralized with 6N HCl, causing precipitation of the KCl. The salt was filtered and the liquid dissolved in CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4, and concentrated to an off-white solid. Yield: 69.65g (0.28 mol, 82%), m.p. 143-144°C. ^1H NMR ($CDCl_3$) δ 7.76 (4H, d, J=8.6Hz), 7.27 (2H, d, J=8.4Hz), 6.90 (2H, d, J=8.8Hz), 6.47 (1H, brs), and 2.38 (3H, s). IR (KBr) 3325, 1900, 1200, 830, 800. Anal. calculated for $C_{13}H_{12}O_3S$ requires: C, 62.89; H, 4.87; S, 12.91. Found: C, 62.90; H, 4.91; S, 12.93.

Preparation of Compound 15

4-(p-Toluenesulfonyl)phenyl benzoate

A solution of Compound 14 (7.64g, 30.77 mmol) in 12 ml pyridine/30 ml $CHCl_3$ at 23°C was treated with benzoyl chloride (4.20 ml, 36.18 mmol). After one hour, the mixture was diluted in $CHCl_3$, washed with water, dried over anhydrous Na_2SO_4, and concentrated. The pale beige solid was azeotroped with toluene to give 9.22g product (26.26 mmol, 85%), which was used

without purification, m.p. 192-198°C. ^{1}H NMR ($CDCl_3$) δ 8.17 (2H, m), 8.01 (2H, d, J=8.8Hz), 7.83 (2H, d, J=8.3Hz), 7.66 (1H, m), 7.52 (2H, t, J=7.7Hz), 7.30-7.37 (4H, m), and 2.41 (3H, s). IR (KBr) 1745, 1200, 1045, 820, 730, 700.

Preparation of Compound 16

4-(p-Toluenesulfonyl)phenyl methyl ether

A mixture of Compound 14 (20.13g, 81.07 mmol), K_2CO_3 (16.61g, 120.18 mmol), and CH_3I (6.20 ml, 99.58 mmol) in 500 ml acetone was refluxed for 4 hours. (An additional 1.00 ml CH_3I (16.00 mmol) was required to drive the reaction to completion.) The mixture was filtered through a diatonaceous earth material sold under the name Celite and concentrated. The yellow-white solid was dissolved in $CHCl_3$, washed with H_2O, then with brine, and dried over anhydrous Na_2SO_4. The volume was reduced, and the compound was triturated with hexane and filtered to give Compound 16 as a white solid, 119.53g (92%). ^{1}H NMR ($CDCl_3$) δ 7.85 (2H, d, J=8.9Hz), 7.79 (2H, d, J=8.3Hz), 7.27 (2H, d, J=8.4Hz), 6.95 (2H, d, J=8.9Hz), 3.83 (3H, s), and 2.38 (3H, s).

Preparation of Compound 7(d)

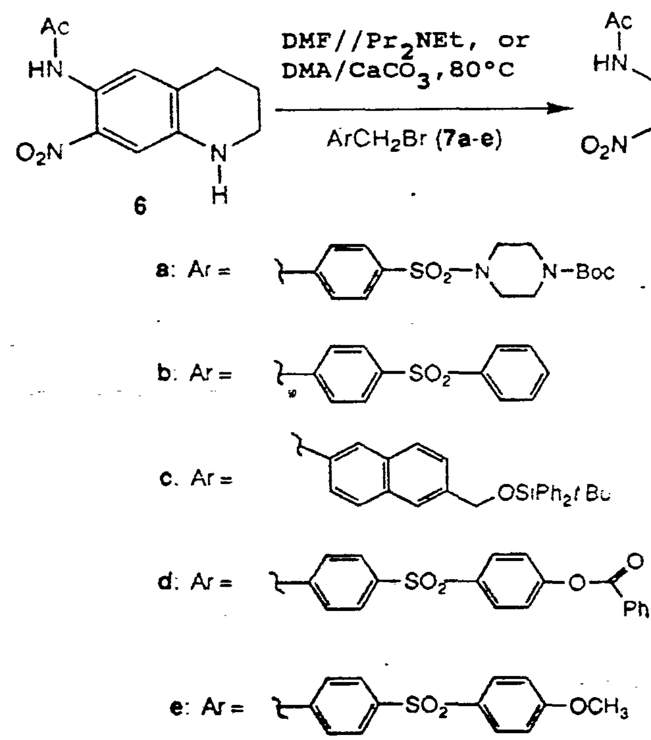
4-(4'-Benzoyloxyphenyl)sulfonylbenzyl bromide

A suspension of 4-(p-toluenesulfonyl)phenyl benzoate (7.43g, 21.08 mmol) and N-bromosuccinimide (3.76g, 21.13 mmol) in 150 ml CCl_4 was heated to reflux under a 200W light. After one hour, ^{1}H NMR of an aliquot showed approximately 53% desired product, along with 30% dibromide and 17% starting material. The mixture was cooled, diluted in CH_2Cl_2, washed with water, dried (anhydrous Na_2SO_4), and concentrated to a yellow-white solid, 8.36g (approximately 4.35g 7(d), 48%). The product was used without purification. ^{1}H NMR ($CDCl_3$) δ 8.19 (2H, m), 7.90-8.05 (4H, m), 7.70 (1H, m), 7.50-7.56 (4H, m), 7.39 (2H, m), and 4.49 (2H, s).

Preparation of Compound 7(e)
4-(4'-methoxyphenyl)sulfonylbenzyl bromide

Compound 16 was brominated as described for Compound 7(d) to give Compound 7(e) in 64% yield. The product was used without purification. ^{1}H NMR ($CDCl_3$) δ 7.86 (4H, m), 7.48 (2H, s, J=8.4Hz), 6.95 (2H, d, J=8.9Hz), 4.44 (2H, s), and 3.83 (3H, s).

Example 5: Preparation of Compounds 8(a) - 8(e)



Preparation of Compound 8(a) -
6-Acylamino-7-nitro-N-[4-(N,N(1-tert-butylcarboxamyl)piperazinylsulfamoylbenzyl]-tetrahydroquinoline

A solution of Compound 6 (0.277g, 1.18 mmol) and bromide 7(a) (0.56g, 1.35 mmol) in 10 ml DMA with dry

$CaCO_3$ (0.184g, 1.84 mmol) was heated to 80°C for 6 hours. The mixture was diluted in ethyl acetate, washed twice with water, dried over anhydrous Na_2SO_4, and concentrated. Purification by flash chromatography (50% EtOAc/Hexane) gave Compound 8(a) as a purple solid, 0.403g (0.70 mmol, 60%), m.p. 190-195°C. ^1H NMR ($CDCl_3$) δ 9.95 (1H, brs), 8.29 (1H, s), 7.71 (2H, d, J=8.3Hz), 7.39 (2H, d, J=8.2Hz), 7.12 (1H, s), 4.57 (2H, s), 3.50 (4H, m), 3.40 (2H, m), 2.98 (4H, m), 2.90 (2H, m), 2.22 (3H, s), 2.04 (2H, m) and 1.40 (9H, s). IR (KBr) 1680, 1510, 1330. HRMS, exact mass calculated for $C_{27}H_{35}N_5O_7S$: M+ required: 573.2257. Found: 573.2257

Preparation of Compound 8(b)

6-Acylamino-7-nitro-N-[4-(phenylsulfonyl)benzyl]tetrahydroquinoline

A solution of Compound 6 (0.402g, 1.17 mmol), bromide 7(b) (0.765g, 2.46 mmol) and diisopropylethylamine (0.44 ml, 2.53 mmol) in 4 ml DMF was heated to 70°C for 5 hours. Workup and purification were as described for 8(a) to provide 8(b) as a red-orange solid, 0.577g (73%), m.p. 202-205°C. ^1H NMR ($CDCl_3$) δ 9.90 (1H, brs), 8.28 (1H, s), 7.89-7.95 (4H, m, 7.50-7.52 (3H, m), 7.35 (2H, d, J=8.2Hz), 7.07 (1H, s), 4.52 (2H, s), 3.36 (2H, m), 2.87 (2H, m), 2.22 (3H, s), and 2.04 (2H, m). HRMS, exact mass calculated for $C_{24}H_{23}N_3O_5S$: M+ requires 465.1358. Found: 465.1373.

Preparation of Compound 8(c)

6-Acylamino-7-nitro-N-[6-tert-butyldiphenylsiloxymethyl)-2-naphthobenzyl]tetrahydroquinoline

Compound 8(c) was prepared from Compounds 6 and 7(c) as described for Compound 8(b) with a 91% yield. ^1H NMR ($CDCl_3$) δ 9.90 (1H, s), 8.28 (1H, s), 7.70-7.82 (8H, m), 7.63 (1H, s), 7.35-7.43 (7H, m), 7.30 (1H, s), 4.91 (2H, s), 4.65 (2H, s), 3.45 (2H, m), 2.90 (2H, m), 2.22 (3H, s), 2.05 (2H, m), and 1.11 (9H, s). IR

(neat) 3360, 1680, 1080 (broad), 880, 820, 750, 700. HRMS, exact mass calculated for $C_{39}H_{41}N_3O_4Si$: M+ requires 643.2866. Found: 643.2822

Preparation of Compound 8(d) - 6-Acylamino-7-nitro-N-[4-(4'-benzoyloxyphenylsulfonyl)benzyl]-tetrahydroquinoline

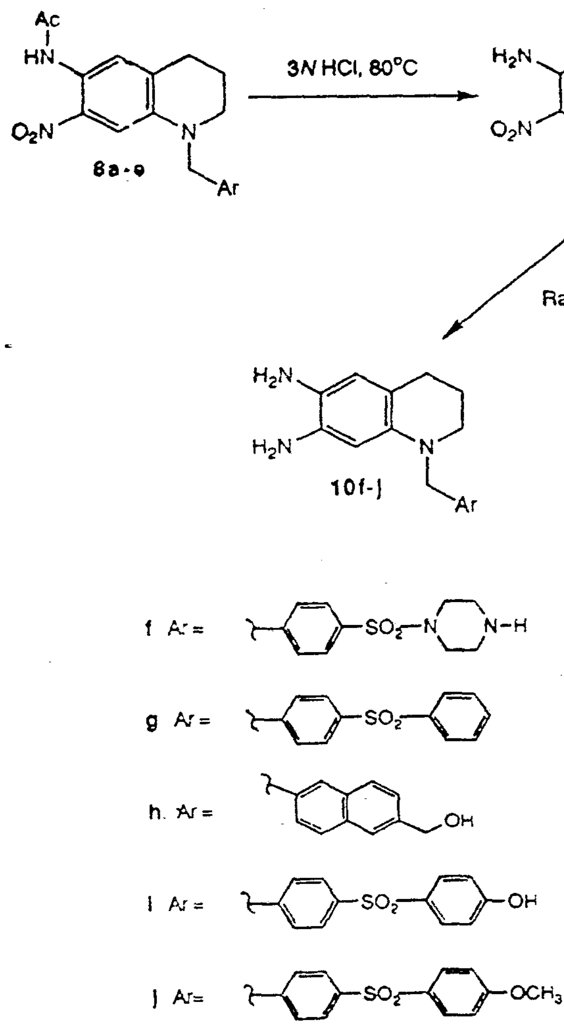
Compound 8(d) was prepared from Compounds 6 and 7(d) as described for Compound 8(b) with a 57% yield, m.p. 174-178. ^1H NMR ($CDCl_3$) δ 9.95 (1H, s), 8.29 (1H, s), 8.16 (2H, d, J=8.3Hz), 8.01 (2H, d, J=8.7Hz), 7.92 (2H, d, J=8.3Hz), 7.65 (1H, m), 7.51 (2H, m), 7.37 (4H, dd, J=8.6, 2.1Hz), 7.12 (1H, s), 4.54 (2H, s), 3.36 (2H, m), 2.88 (2H, m), 2.22 (3H, s), and 2.04 (2H, m). IR (KBr) 3360, 1730, 1670, 1570, 1150, 880, 830, 810, 700. HRMS, exact mass calculated for $C_{31}H_{27}N_3O_7S$: M+ requires 585.1570. Found: 585.1567.

Preparation of Compound 8(e) - 6-Acylamino-7-nitro-N-[4-(4'-methoxyphenylsulfonyl)-benzyl]tetrahydroquinoline

Compound 8(e) was prepared from 6 and 7(e) in 82% yield as described for 8(b), m.p. 168-171°C. ^1H NMR ($CDCl_3$) δ 8.30 (1H, s), 7.86 (4H, d, J=8.9Hz), 7.33 (2H, d, J=8.4Hz), 7.08 (1H, s), 6.96 (2H, d, J=8.9Hz), 4.52 (2H, s), 3.83 (3H, s), 3.37 (2H, m), 2.87 (2H, m), 2.22 (3H, s), and 2.01 (2H, m). Analytical data calculated for $C_{25}H_{25}N_3O_6S$ requires: C, 0.59; H, 5.09; N, 8.48. Found: C, 60.51; H, 5.12; N, 8.37.

<u>Example 5: Preparation of Compounds 9(f) - 9(j) and Compounds 10(f) - 10(j)</u>

Compounds 9(f) - 9(j) and 10(f) - 10(j) were prepared according to the following reaction scheme:



Preparation of Compounds 9(f) - 9(j) - 6-Amino-7-nitro-N-[4-(N,N-piperazinylsulfamoyl)benzyl]-tetrahydroquinoline, 6-Amino-N-[4(phenylsulfonyl)-benzyl]tetrahydroquinoline, 6-Amino-7-nitro-N-[(6-hydroxymethyl)-2-naphthobenzyl]tetrahydroquinoline, 6-Amino-7-nitro-N-[4-(4'-hydroxyphenylsulfonyl)-benzyl]tetrahydroquinoline, and 6-Amino-7-nitro-N-[4-(4'-methoxyphenylsulfonyl)benzyl]tetrahydroquinoline

The following procedure, described for Compound 9(f), is representative: A suspension of Compound 8(a) (0.4036g, 0.70 mmol) in 3N HCl (12 ml) was heated to

60°C for 5.5 hours. The mixture was poured into 100 ml saturated $NaHCO_3$, extracted 4 times with ethyl acetate, dried over anhydrous Na_2SO_4, and concentrated. Purification by flash chromatography (10% EtOH/$CHCl_3$) yielded 0.215g Compound 9(f) as a purple solid (0.50 mmol, 71%), m.p. 194-196°C. ^1H NMR ($CDCl_3$) δ 7.71 (2H, d, J=8.2Hz), 7.42 (2H, d, J=8.1Hz), 7.06 (1H, s), 6.52 (1H, s), 5.67 (2H, brs), 4.50 (2H, s), 3.31 (2H, t, J=5.7Hz), 2.95-2.99 (4H, m), 2.90-2.93 (4H, m), 2.80 (2H, t, J=6.0Hz), and 2.01-2.03 (2H, m). IR (KBr) 3460, 3420, 1240. HRMS, exact mass calculated for $C_{20}H_{23}N_3O_4S$: M+ requires 431.1627. Found: 431.1626.

9(g): ^1H NMR ($CDCl_3$) δ 7.88-7.94 (4H, m), 7.50-7.54 (3H, m), 7.38 (2H, d, J=8.3Hz), 7.02 (1H, s), 6.50 (1H, s), 5.65 (2H, brs), 4.46 (2H, s), 3.25-3.27 (2H, m), 2.75-2.77 (2H, m), and 1.90-2.04 (2H,m).

9(h): ^1H NMR ($CDCl_3$) δ 7.79 (3H, m), 7.68 (1H, s), 7.39-7.48 (2H, m), 7.25 (1H, s), 6.51 (1H, s), 5.63 (2H, brs), 4.85 (2H, d, J=5.9Hz), 4.58 (2H, s), 3.33 (2H, t, J=5.7Hz), 2.79 (2H, t, J=6.3Hz), 2.04 (2H, m), and 1.71 (1H, t, J=6.0Hz). IR (KBr) 3480, 3320, 2910, 1560, 880, 810. HRMS, exact mass calculated for $C_{21}H_{21}N_5O_3$: M+ requires 363.1583. Found: 343.1574.

9(i): m.p. 200-250°C; ^1H NMR ($CDCl_3$) δ 7.80-7.86 (4H, m), 7.37 (2H, d, J=8.2Hz), 7.01 (1H, s), 6.90 (2H, d, J=8.7Hz), 6.50 (1H, s), 5.66 (2H, brs), 4.46 (2H, s), 3.28 (2H, t, H=5.7Hz), 2.77 (2H, t, J=6.2Hz), and 1.99 (2H, m). IR (KBr) 3460, 3380, 2940, 1570, 1150, 880, 840. HRMS, exact mass calculated for $C_{22}H_{21}N_3O_5S$: M+ requires 439.1202. Found: 439.1213.

9(j): m.p. 141-143°C; ^1H NMR ($CDCl_3$) δ 7.86 (4H, d, J=8.9Hz), 7.35 (2H, d, J=8.3Hz), 7.03 (1H, s), 6.96 (2H, d, J=8.9Hz), 6.50 (1H, s), 5.65 (2H, brs),

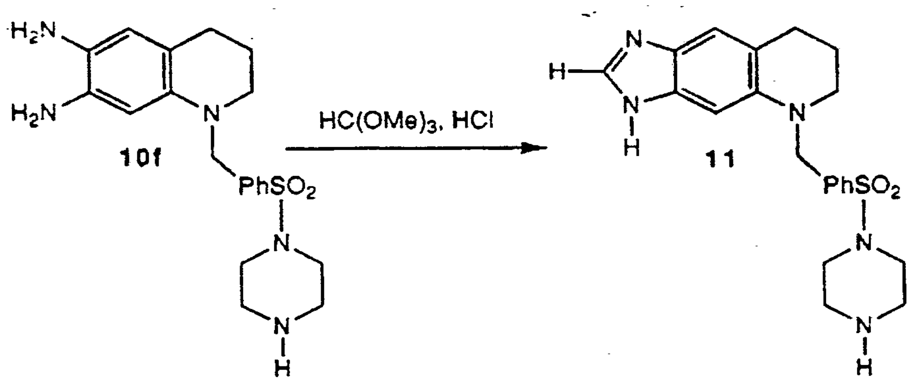
4.45 (2H, s), 3.83 (3H, s), 3.26 (2H, t, J=5.8Hz), 2.77 (2H, m), 1.98 (2H, m). IR (KBr) 3400, 1500, 1250, 1150, 1100, 860, 800.

Preparation of Compounds 10(f)-10(j) - 6,7-Diamino-N-[4-(N,N-piperazinylsulfamoyl)benzyl]-tetrahydroquinoline, 6,7-Diamino-N-[4-(phenylsulfonyl)benzyl]-tetrahydroquinoline, 6,7-Diamino-N-[(6-hydroxymethyl)-2-naphthobenzyl]tetrahydroquinoline, 6,7-Diamino-N-[4-4'-hydroxyphenylsulfonyl)-benzyl]tetrahydroquinoline, and 6,7-Diamino-N-[4-4'-methoxyphenylsulfonyl)-benzyl]tetrahydroquinoline

The following procedure described for Compound 10(f) is representative: A solution of Compound 9(f) (0.215g, 0.50 mmol) in 6 ml MeOH/2 ml THF over a catalytic amount of Raney Ni/H_2O was heated to reflux and treated with anhydrous hydrazine (1.00 ml, 31.54 mmol). After one hour, during which loss of color occurred, the mixture was filtered through a diatomaceous earth material sold under the trade name Celite, concentrated, and azeotroped with CH_3CN/benzene to give 0.158g of Compound 10(a) (0.39 mmol, 79%). In all cases, the diamine was used immediately for the cyclization reaction. ^1H NMR ($CDCl_3$) δ 7.69 (2H, d, J=8.2Hz), 7.44 (2H, d, J=8.2Hz), 6.44 (1H, s), 5.85 (1H, s), 4.42 (2H, s), 3.24 (2H, m), 2.97 (4H, m), 2.93 (4H, m), 2.70 (2H, m), and 1.98 (2H, m).

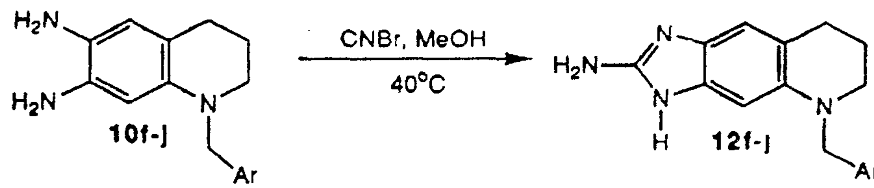
<u>Example 6: Preparation of Compound 11 and Compounds 12(f)-12(j) of the Invention</u>

Preparation of Compound 11 - 6-7-Imidazol-N-[4-(N,N-piperazinylsulfamoyl)-benzyl]tetrahydroquinoline



A solution of diamine Compound 10(f) (0.084 g, 0.21 mmol) in 2 ml $HC(OCH_3)_3$ at 23°C was treated with one drop of concentrated HCl, causing the formation of a precipitate. The mixture was stirred for one hour, poured into saturated $NaHCO_3$ solution (20 ml), and extracted with 50 ml $CHCl_3$. The organic layer was dried with anhydrous Na_2SO_4, concentrated, and purified by flash chromatography (1.5% NH_3/13.5% EtOH/85% $CHCl_3$) to give Compound 11 as a yellow-orange solid (0.032g, 0.08 mmol, 38%). ^1H NMR (acetone-d_6) δ 8.01 (1H, s), 7.76 (1H, s), 7.70 (2H, d, J=8.1Hz), 7.56 (2H, d, J=8.1Hz), 7.20 (1H, s), 6.52 (1H, s), 4.64 (2H, s), 3.45 (2H, t, J=5.7Hz), 2.92 (2H, t, J=6.1Hz), 2.84 (4H, brs), 2.81 (4H, brs), and 2.04 (2H, m). HRMS, exact mass calculated for $C_{21}H_{25}N_5O_2S$: M+ requires 411.1729. Found: 411.1719.

Preparation of Compounds 12(f) - 12(j) - 6,7-(2-Aminoimidazol)-N-[4-(N,N-piperazinylsulfamoyl)benzyl]tetrahydroquinoline, 6,7-(2-Aminoimidazol)-N-[4-(phenylsulfonyl)benzyl]-tetrahydroquinoline, 6,7-(2-Aminoimidazol)-N-[(6-hydroxymethyl)-2-naphthobenzyl]tetrahydroquinoline, 6,7-(2-Aminoimidazol)-N-[4-(4'-hydroxyphenylsulfonyl)benzyl]tetrahydroquinoline, and 6,7-(2-Aminoimidazol)-N-[4-(4'-methoxyphenylsulfonyl)benzyl]tetrahydroquinoline



The following procedure, described for 12(i), is representative: A solution of diamine Compound 10(i) (1.59 mmol) in 6 ml CH_3CN/2 ml MeOH was heated to 70°C and treated with 5.0M CNBr/CH_3CN solution (0.5 ml, 2.5 mmol). After two hours, the mixture was diluted in 0.5N HCl and extracted twice with ethyl acetate. The aqueous layer was neutralized with 6N NaOH, saturated with NaCl, and extracted twice with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to a brown solid. Purification by flash chromatography (5% to 10% EtOH/CH_2Cl_2) gave Compound 12(i) as a light brown solid, 0.173g (0.40 mmol, 25%), m.p. 230-245°C. ^{1}H NMR (DMSO-d_6) δ 7.86 (3H, m), 7.75 (2H, d, J=8.7Hz), 7.43 (2H, d, J=8.2Hz), 6.91 (3H, m), 6.16 (1H, s), 4.53 (2H, s), 3.35 (2H, m), 2.80 (2H, m) and 1.90 (2H, m). IR (KBr) 3400 (broad), 1660, 1280. HRMS, exact mass calculated for $C_{23}H_{22}N_4O_3S$: M+ requires 434.1412. Found: 434.1401. ^{13}C NMR (DMSO-d_6) δ 162.3, 149.5, 144.8, 141.5, 140.7, 130.7, 129.7, 129.2, 127.5, 127.4, 127.2, 120.3, 118.0, 116.2, 111.5, 93.3, 54.7, 49.9, and 28.3.

12(f): ^{1}H NMR ($CDCl_3$) δ 7.65 (2H, d, J=8.3Hz), 7.48 (2H, d, J=8.2Hz), 6.93 (1H, s), 6.10 (1H, s), 4.52 (2H, s), 3.43 (2H, t, J=5.5Hz), 3.29 (4H, m), 3.04 (4H, m), 2.92 (2H, t, J=6.5Hz), and 2.17 (2H, m). IR (KBr) 3420, 1610. HRMS, exact mass calculated for $C_{21}H_{26}N_6O_2S$: M+ requires 426.1833. Found: 427.1940.

12(g): ^{1}H NMR ($CDCl_3$) δ 7.92 (2H, d, J=8.3Hz), 7.83 (2H, d, J=8.3Hz), 7.47-7.55 (3H, m), 7.39 (2H, m), 6.89 (1H, s), 6.23 (1H, s), 4.44 (2H, s), 3.32 (2H, m), 2.85 (2H, m), and 2.01 (2H, m). IR (film) 1630, 1290, 1150, 820. HRMS, exact mass calculated for $C_{23}H_{22}N_4O_2S$: M+ required 418.1463. Found: 418.1467.

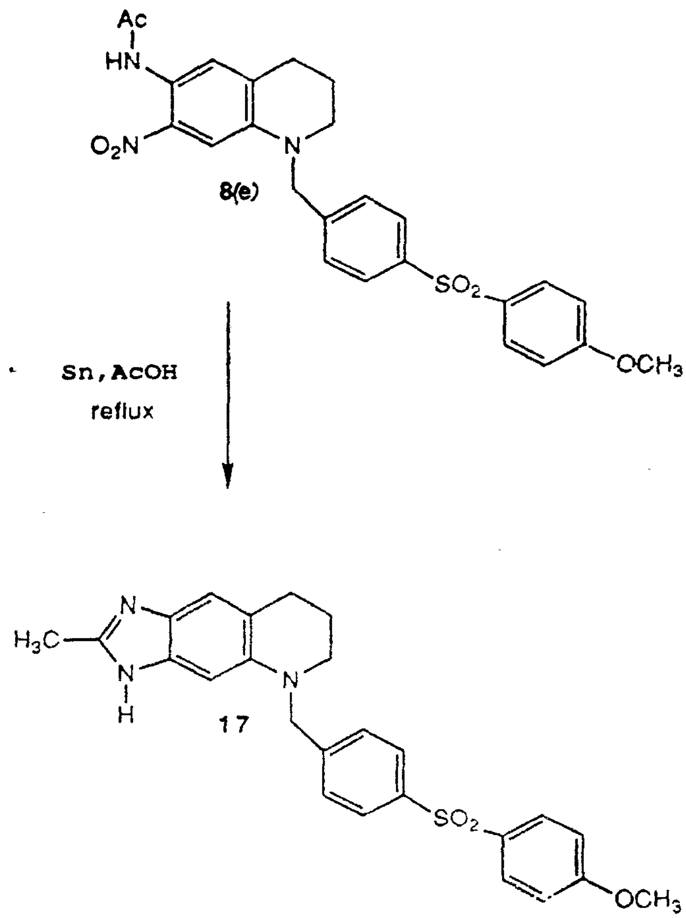
12(h): ^{1}H NMR (DMSO-d_6) δ 7.71-7.84 (4H, m), 7.41 (2H, m), 6.68 (1H, s), 6.30 (1H, s), 5.74 (2H, brs), 4.62 (2H, s), 4.54 (2H, s), 3.32 (2H, m), 2.77 (2H, m), and 1.93 (2H, m). IR (KBr) 3440, 1720. HRMS, exact mass calculated for $C_{22}H_{22}N_4O$: M+ requires 358.1793. Found: 358.1814.

12(j): m.p. 217-228°C; ^{1}H NMR (DMSO-d_6) δ 7.84 (4H, d, J=8.9Hz), 7.46 (2H, d, J=8.3Hz), 7.09 (2H, d, J=8.9Hz), 6.66 (1H, s), 6.10 (1H, s), 5.74 (2H, brs), 4.45 (2H, s), 3.80 (3H, s), 3.15-3.26 (2H, m), 2.74 (2H, m), and 1.83 (2H, m). IR (KBr) 3400, 2950, 1650, 1600, 1400, 1140, 1100, 830, 800. HRMS, exact mass calculated for $C_{24}H_{24}N_4O_3S$: M+ requires 448.1569. Found: 448.1559.

Example 7: Preparation of Compound 17

6,7-(2-Methylimidazol)-N-[4-(4'-methoxyphenylsulfonyl)benzyl]-tetrahydroquinoline

Compound 17 was prepared by the following reaction:



A solution of compound 8(e) (0.1131g, 0.228 mmol) in 3 ml glacial acetic acid was treated with Sn° (0.1443g, 1.216 mmol) and refluxed overnight. The mixture was filtered through a diatomaceous earth material sold under the trade name Celite, neutralized with saturated $NaHCO_3$ solution, and extracted twice with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and purified by flash chromatography (10% $MeOH/CH_2Cl_2$) to give Compound 17 as a light yellow solid, 0.0724g (0.162 mmol, 71%), m.p. 162-165°C (decomposition). Exact mass calculated for $C_{25}H_{25}N_3O_3S$: 447.1616. Found: 447.1586. IR (KBr) 3400, 1590, 1250, 1140. ^1H NMR ($CDCl_3$) δ 7.84 (4H, m), 7.38 (2H, d, J=8.3Hz), 7.16 (1H, s), 6.96 (2H, d, J=8.9Hz), 6.33 (1H, s), 4.49 (2H, s), 3.83 (3H, s), 3.38 (2H, m), 2.92 (2H, m), 2.47 (3H, s), and 2.04 (2H, m).

Example 8: Determination of Inhibition Constants Against 5,10-Methylene-tetrahydrofolate

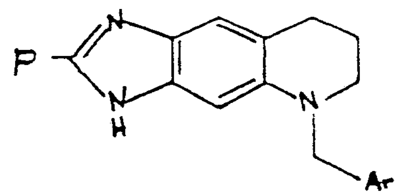
Thymidylate synthase inhibition constants K_i have been determined by the following method. All assays were run at 25°C and initiated by the addition of three different types of thymidylate synthase ("TS"): (1) Escherichia coli TS ("ETS"); (2) Candida TS ("CTS"), which is from a fungus; and (3) human TS ("HTS").

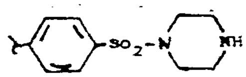
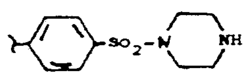
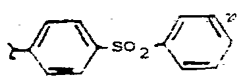
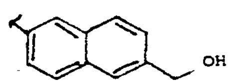
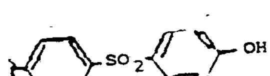
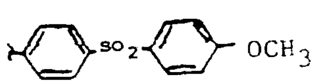
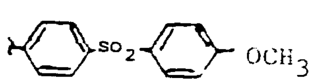
TS exhibits ordered bireactant kinetics (Daron, H.H. and Aull, J.L., J. Biol. Chem. 253, 940-9451 (1978), and the dUMP (2'-deoxyuridine-5'-monophosphate) concentration used for these reactions was near saturation levels so that the assays were pseudo-single substrate. All reaction mixtures contained 50 mM Tris at pH 7.8 (the final pH of the reaction was 7.6), 10 mM DTT (dithiothreitol), 1 mM EDTA (ethylenediaminetetraacetic acid), 25 mM $mgCl_2$, 15 mM H_2CO (formaldehyde) and 25 microM dUMP. When human TS was assayed, 100 micrograms/ml of BSA (bovine serum albumin) was present in the reactions. The range of THF (tetrahydrofolate) was 5 to 150 microM (eight concentrations: 5, 6.6, 10, 13, 16, 25, 50 and 150 microM). A standard curve in the absence of inhibitor was run with each experiment. Three curves were then run with inhibitor at three different concentrations with a minimal range, where possible, from 1/2 to 2 times the K_i (Cleland, W.W., Biochim. Biophys. Acta 67, 173-187 (1963). These assays were done on a spectrophotometer at 340 nm (Wahba, A.J. and Friedkin, M., J. Biol. Chem. 236, PC11-PC12 (1961), following the formation of DHF (dihydrofolate) (mM extinction coefficient of 6.4) or by following the release of tritium (Lomax, M.I.S. and Greenberg, G.R., J. Biol. Chem. 242, 109-113 (1967) from the 5-position of dUMP (assays for tritium release contained 0.5 microcI dUMP). Charcoal was used to remove unreacted dUMP from the tritium release reaction mixtures, and the

resultant water was counted to determine the extent of reaction. Inhibition constants were then determined by plotting the apparent K_m or the reciprocal of the apparent V_{max} against the inhibitor concentration (Cleland, W.W., The Enzymes, 2, 1-65 (1970)).

The results obtained are tabulated in Table I:

TABLE I



Cmpd #	P	Ar	K_i (μM) ETS	HTS	CTS
11	-H		9	15	38
12(f)	$-NH_2$		0.24	0.17	0.25
12(g)	$-NH_2$		0.062	0.048	0.1
12(h)	$-NH_2$		0.041	0.074	0.086
12(i)	$-NH_2$		0.018	0.013	0.007
17	$-CH_3$		2.4	1.5	1.7
12j	$-NH_2$		0.069	0.033	0.030

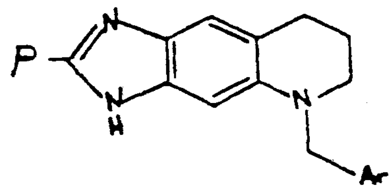
Example 9: In Vitro Testing

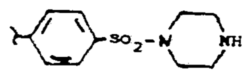
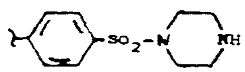
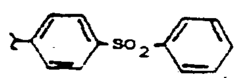
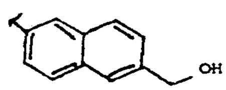
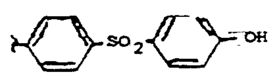
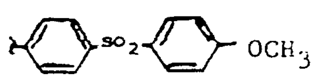
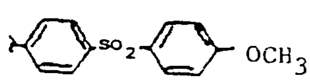
Cellular growth in the presence of the compounds in question was assessed using three cell lines: (1) the L1210 murine leukemia (ATCC CCL 219); (2) CCRF-CEM, a human lymphoblastic leukemia line of T-cell origin (ATCC CCL 119); and (3) a thymidine kinase deficient human adenocarcinoma line (GC_3/M TK). Both lines were maintained in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum without antibiotics.

IC_{50} values were determined in 150 microliter microcultures each containing 1500 (L1210), 4000 (CCRF-CEM) or 10,000 (GC_3/M TK) cells established in 96 well plates in growth medium supplemented with 50 IU/ml penicillin and 50 mcg/ml streptomycin. Growth was measured over 3 days (L1210) or 5 days (CCRF-CEM and GC_3/M TK) of continuous exposure to varying concentrations of each test compound added 4 hours after initial cell plating by the MTT-tetrazolium reduction assay of Mosmann, T.J. Immunol. Meth., 65, 55-63 (1983), modified according to Alley et al., Cancer Res. 48, 589-601 (1988). Water insoluble derivatives were dissolved in DMSO and diluted as a final concentration of 0.3% solvent in cell cultures.

The results obtained from this procedure are tabulated below in Table II.

TABLE II

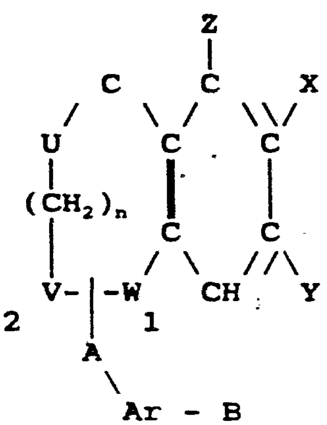


Cmpd #	P	Ar	L1210	CCRFCEM	GC3-M
11	-H		20	11	43
12(f)	$-NH_2$		2.05	2.2	>50
12(g)	$-NH_2$		2.0	3.9	>5
12(h)	$-NH_2$		3.1	>5	>5
12(i)	$-NH_2$		12	22	>50
17	$-CH_3$		2.8	8.0	4.1
12(j)	$-NH_2$		2.6	5.1	9.0

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

I CLAIM:

1. A compound capable of inhibiting thymidylate synthase having the formula:



wherein:

X and Y form a five- or six-membered heterocyclic ring containing at least one nitrogen;

Z is a hydrogen, halogen, carbon, oxygen or nitrogen atom;

U is a carbon or nitrogen atom;

n is 0 or the integer 1;

V is a carbon or nitrogen atom;

W is a carbon or nitrogen atom;

A is in the 1- or 2-position and is a nitrogen atom, a sulfur atom, or a substituted or unsubstituted alkylene group;

Ar is an aryl or heteroaryl group having one or more rings; and

B is either (i) an oxygen or nitrogen atom, or a -CO- or $-SO_2-$ group, any of which is linked to an amino acid, aryl group, heterocyclic group or alkyl group, or (ii) a substituted or unsubstituted alkyl group,

with the proviso that, if A is sulfur, both V and W are carbon atoms.

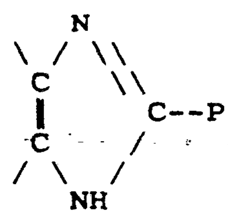
2. The compound of claim 1 wherein said compound has a thymidylate synthase inhibition constant K_i of less than or equal to about 10^{-4}M.

3. The compound of claim 1 wherein the K_i value is less than or equal to about 10^{-6}M.

4. The compound of claim 1 wherein the K_i value is less than or equal to about 10^{-9}M.

5. The compound of claim 1 wherein X and Y form a heterocyclic ring selected from the group consisting of pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine and pyridazine rings.

6. The compound of claim 1 wherein X and Y form the ring:



wherein P is a lower alkyl group, an amino group or hydrogen.

7. The compound of claim 1 wherein Z is hydrogen.

8. The compound of claim 1 wherein n is O.

9. The compound of claim 1 wherein A is a substituted or unsubstituted alkylene group.

10. The compound of claim 1 wherein Ar is a phenyl or a naphthyl group.

11. The compound of claim 1 wherein B is an $-SO_2$ group linked to an amino acid, an aryl group or an alkyl group.

12. The compound of claim 1 wherein X and Y form the ring:

```
        N
   \  /  \\
    C      \\
    ‖        C--P
    C       /
   / \    /
       NH
```

wherein P is a lower alkyl group, an amino group or hydrogen;

U is carbon;

Z is hydrogen;

n is 0;

V is carbon;

W is nitrogen;

A is methylene; and

Ar-B is selected from the group consisting of:

```
      CH - CH                 CH2-CH2
     //      \\              /        \
 - C           C - SO2 - N              NH     ;
     \       /              \         /
      CH = CH                 CH2-CH2


      CH - CH                 CH - CH
     //      \\              //      \\
-- C           C - SO2 - C             CH      ;
     \       /              \        /
      CH = CH                 CH = CH


      CH - CH                 CH - CH
     //      \\              //      \\
-- C           C - SO2 - C             C-OH    ;
     \       /              \        /
      CH = CH                 CH = CH


      CH - CH                 CH - CH
     //      \\              //      \\
-- C           C - SO2 - C             C-OCH3  ;
     \       /              \        /
      CH = CH                 CH = CH
```

```
      CH     CH
 \  /   \\  /  \\
  C       C     CH
  ‖       |     |
  CH      C     C--CH2-OH                 ;
   \    // \   //
     CH      CH
```

```
       CH - CH           COOH
      //      \\           |
  -- C          C - CO-NH-CH-CH2-CH2-COOH   ;
      \        /
       CH = CH
```

```
        CH     CH
  \  //   \  /   \\
   C        C      C - O-R wherein R is H or
   |        ‖      |         alkyl, such as
   S  ---   C      CH        methyl; and
             \   //
               CH
```

```
       CH      CH2-OH
  \  /    \\  /
   C        C
   ‖        |
   CH       C
     \    //  \
       C       T wherein T is H or -CN.
       |
       CH2-OH
```

13. The compound of claim 12 wherein P is hydrogen and Ar-B is

```
       CH - CH              CH2-CH2
      //      \\           /       \
   - C          C - SO2 - N          NH    .
      \        /           \       /
       CH = CH              CH2-CH2
```

14. The compound of claim 12 wherein P is $-NH_2$ and Ar-B is

```
       CH - CH              CH2-CH2
      //      \\           /       \
   - C          C - SO2 - N          NH    .
      \        /           \       /
       CH = CH              CH2-CH2
```

15. The compound of claim 12 wherein P is $-NH_2$ and Ar-B is

```
       CH - CH             CH - CH
      //      \\          //      \\
  -- C          C - SO2 - C          CH     .
      \        /           \        /
       CH = CH              CH = CH
```

16. The compound of claim 12 wherein P is $-NH_2$ and Ar-B is

```
     CH      CH
 \ /   \\ /    \\
  C      C      CH
  |      |      |
  CH     C      C--CH2-OH                  .
   \   // \   //
     CH      CH
```

17. The compound of claim 12 wherein P is $-NH_2$ and Ar-B is

```
       CH - CH             CH - CH
      //      \\          //      \\
  -- C          C - SO2 - C          C-OH   .
      \        /           \        /
       CH = CH              CH = CH
```

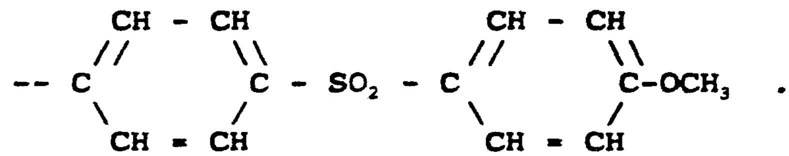
18. The compound of claim 12 wherein P is $-CH_3$ and Ar-B is

```
       CH - CH             CH - CH
      //      \\          //      \\
  -- C          C - SO2 - C          C-OCH3  .
      \        /           \        /
       CH = CH              CH = CH
```

19. The compound of claim 12 wherein P is $-NH_2$ and Ar-B is

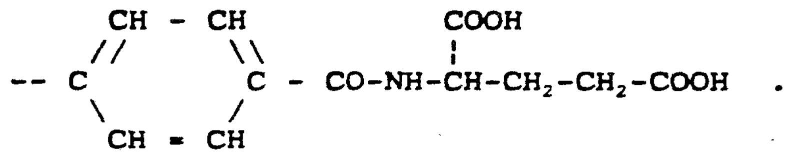
```
       CH - CH             CH - CH
      //      \\          //      \\
  -- C          C - SO2 - C          C-OCH3  .
      \        /           \        /
       CH = CH              CH = CH
```

20. The compound of claim 12 wherein P is $-NHCH_3$ and -Ar-B is



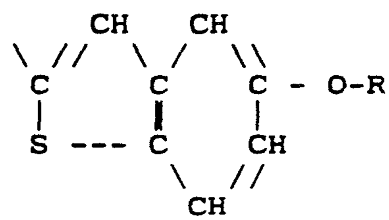
.

21. The compound of claim 12 wherein P is $-NH_2$ and -Ar-B is



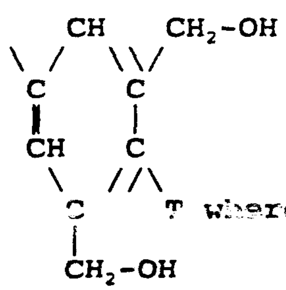
.

22. The compound of claim 12 wherein P is $-NH_2$ and -Ar-B is



wherein R is hydrogen or a methyl group.

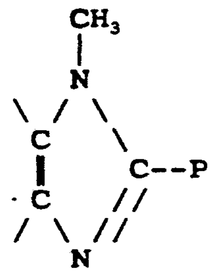
23. The compound of claim 12 wherein P is $-NH_2$ and -Ar-B is



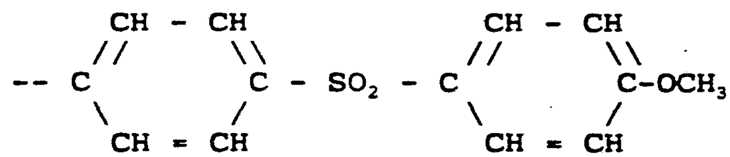
wherein T is H or -CN.

wherein T is hydrogen or -CN group.

24. A compound of claim 1 wherein X and Y form the ring:

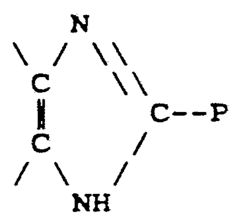


wherein P is selected from the group consisting of $-NH_2$ and methyl; U is carbon; n is 0; V is carbon; W is nitrogen; A is methylene; and -Ar-B is

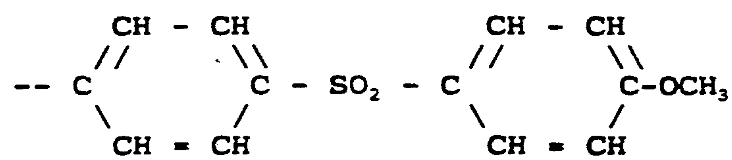


.

25. The compound of claim wherein X and Y form the ring:

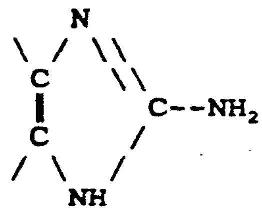


wherein P is selected from the group consisting of -NHOH and $-NHNH_2$; U is carbon; n is 0; V is carbon; W is nitrogen; A is methylene; and -Ar-B is



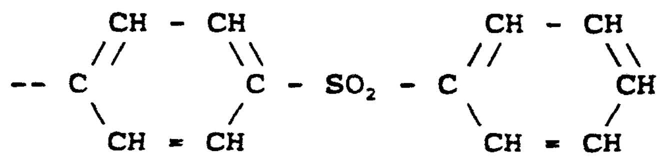
.

26. A compound of claim 1 wherein X and Y form the ring:



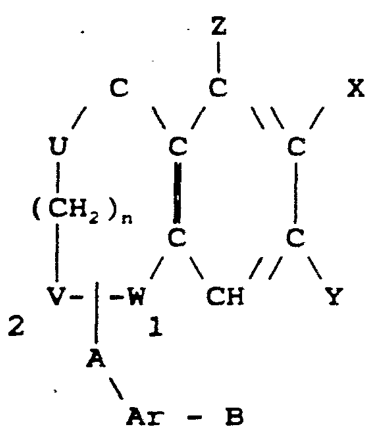
;

U is carbon; n is O; V is carbon; W is carbon; A is divalent sulfur; and -Ar-B is



.

27. A process for making a compound capable of inhibiting thymidylate synthase having the formula:



wherein:

X and Y form a five- or six-membered heterocyclic ring containing at least one nitrogen atom;

Z is a hydrogen, halogen, carbon, oxygen or nitrogen atom;

U is a carbon or nitrogen atom;

n is O or the integer 1;

V is a carbon or nitrogen atom;

W is a carbon or nitrogen atom;

A is in the 1- or 2-position and is a nitrogen atom, a sulfur atom, or a substituted or unsubstituted alkylene group;

Ar is an aryl or heteroaryl group having one or more rings; and

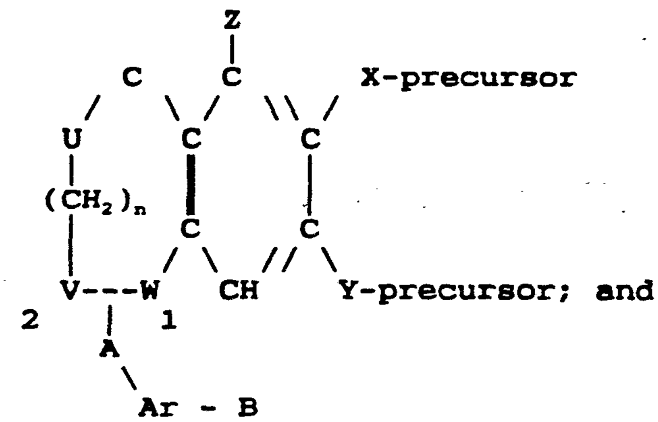
B is (i) either an oxygen or nitrogen atom, or a -CO- or $-SO_2-$ group, any of which is linked to an amino acid, aryl group or alkyl group, or (ii) a substituted or unsubstituted alkyl group,

with the proviso that, if A is sulfur, both V and W are carbon atoms comprising the steps of:

(1) allowing a compound of the formula B-Ar-A-D, wherein D is a displaceable group, to react with a compound of Formula (I):

```
                Z
                |
        C       C          X-precursor
       /   \  /   \\  /
      U      C       C
      |      ‖       |
   (CH2)n    ‖       |              (I)
      |      C       C
      |     / \    // \
      V---W    CH      Y-precursor;
```

wherein X-precursor and Y-precursor are groups which, when cyclized with each other, form a five- or six-membered heterocyclic ring containing at least one nitrogen, to form a substituted compound having the formula:



(2) cyclizing X-precursor and Y-precursor to form with each other a five- or six-membered heterocyclic ring containing at least one nitrogen atom,

provided that one or more of X-precursor, Y-precursor, U, V and W may contain a chemical group or groups which, either before, after or during the course of either the substitution step (1) or the cyclization step (2):

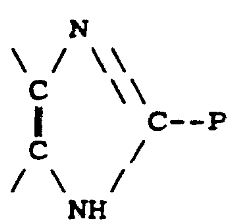
(a) may be protected by a protecting group or

(b) may have one or more of any protecting groups present removed.

28. The process of claim 27 wherein the compound capable of inhibiting thymidylate synthase has a thymidylate synthase inhibition constant K_i of less than or equal to about 10^{-4}.

29. The process of claim 27 wherein X and Y form a heterocyclic ring selected from the group consisting of pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine and pyridazine rings.

30. The process of claim 27 wherein X and Y form the ring:



wherein P is a lower alkyl group, an amino group or hydrogen.

31. The process of claim 27 wherein Z is hydrogen.

32. The process of claim 27 wherein n is O.

33. The process of claim 27 wherein A is substituted or unsubstituted alkylene group.

34. The process of claim 27 wherein Ar is a phenyl or naphthyl group.

35. The process of claim 27 wherein B is an $-SO_2$ group linked to an amino acid, aryl group or alkyl group.

36. The process of claim 27 wherein Ar-B is selected from the group consisting of:

```
      CH - CH                    CH2-CH2
     //      \\                 /       \
 - C          C - SO2 - N              NH      ;
     \       /                  \       /
      CH = CH                    CH2-CH2

      CH - CH                  CH - CH
     //      \\               //      \\
-- C          C - SO2 - C              CH      ;
     \       /               \        /
      CH = CH                  CH = CH

      CH - CH                  CH - CH
     //      \\               //      \\
-- C          C - SO2 - C              C-OH    ;
     \       /               \        /
      CH = CH                  CH = CH

      CH - CH                  CH - CH
     //      \\               //      \\
-- C          C - SO2 - C              C-OCH3   ;
     \       /               \        /
      CH = CH                  CH = CH
```

```
     CH      CH
 \ /   \\ /    \\
  C      C      CH
  ‖      |      |
  CH     C      C--CH2-OH                    ;
   \  // \    //
    CH      CH
```

```
        CH - CH             COOH
       //      \\             |
   -- C          C - CO-NH-CH-CH2-CH2-COOH   ;
       \        /
        CH = CH
```

```
      CH     CH
 \ //   \ /    \\
  C      C      C - O-R wherein R is H or
  |      ‖      |       alkyl, such as
  S ---  C      CH      methyl; and
          \   //
            CH
```

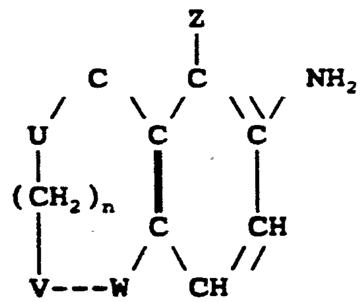
```
     CH     CH2-OH
 \ /    \\/
  C      C
  ‖      |
  CH     C
   \   //\
     C     T wherein T is H or -CN.
     |
     CH2-OH
```

37. The process of claim 30 wherein the compound of Formula I has the structure of Formula (II):

```
               Z
               |
        C      C        NH-Ac
      /   \  /   \\  /
    U       C       C
    |       ‖       |
  (CH2)n    |       |          (II)
    |       C       C
    |     /  \   //  \
    V---W     CH      NO2
```

wherein Ac is a CH_3CO- protective group and is prepared by:

(1) selectively protecting an amine compound of Formula III:



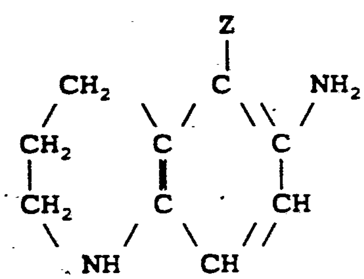
(III)

to form the corresponding acetamide; and

(2) nitrating the corresponding acetamide to form the compound of Formula (II).

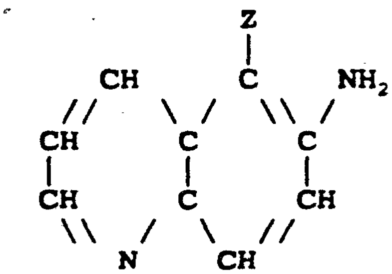
38. The process of claim 37 wherein U is a carbon atom, n is 0, V is a carbon atom, and W is a nitrogen atom.

39. The process of claim 38 wherein the amine of Formula (III) has the formula:



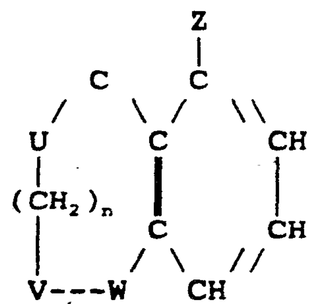
40. The process of claim 37 wherein the amine of Formula (III) is prepared by reducing a compound corresponding to the amine having one or more sites of unsaturation in the 6- or 7-membered ring formed by U, V and W taken in combination with an appropriate number of carbon and hydrogen atoms.

41. The process of claim 40 wherein said compound corresponding to the amine having one or more sites of unsaturation has the formula:



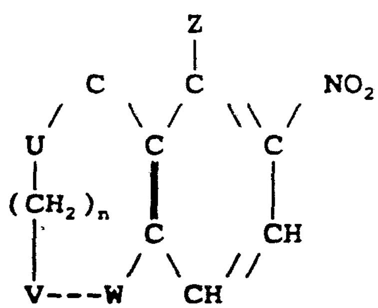
42. The process of claim 37 wherein the amine of Formula (III) is prepared by:

(1) nitrating an unsubstituted compound of Formula (IV):



(IV)

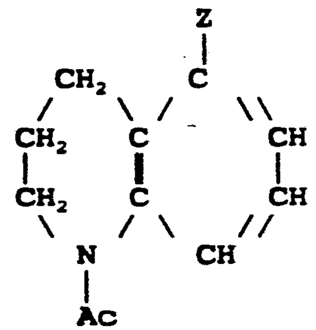
to form a nitrated compound having the formula:



; and

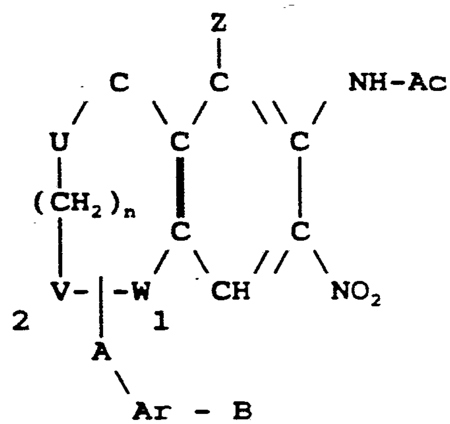
(2) reducing the nitrated compound to form the amine of Formula (III).

43. The process of claim 42 wherein the compound of Formula (IV) has the formula:



wherein Ac is a CH_3CO- protective group which is removed between the nitration step and the reduction step.

44. The process of claim 27 wherein, after step (1), the compound

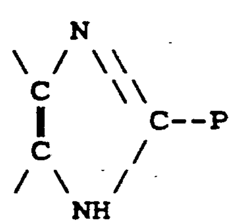


is treated with

(1) a deprotecting agent to convert the -NH-Ac group to a free amino group,

(2) a reducing agent to convert the $-NO_2$ group to an amino group, and

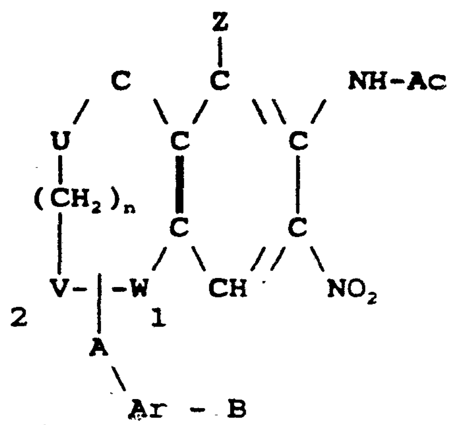
(3) a cyclizing agent to form a ring having the formula:



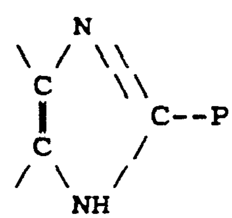
wherein P is lower alkyl, amino or hydrogen.

45. The process of claim 44 wherein the cyclizing agent is $HC(OCH_3)_3$ or CNBr.

46. The process of claim 27 wherein, after step (1), the compound

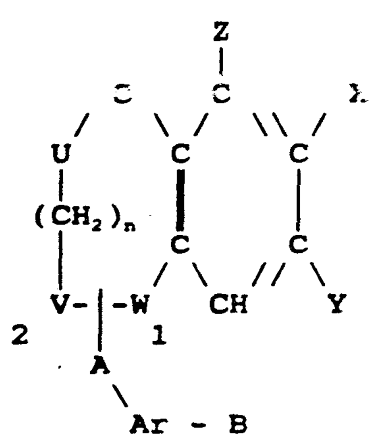


is treated with tin metal in the presence of acetic acid to form a ring having the formula:



wherein P is a methyl group.

47. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier in combination with an amount effective to inhibit thymidylate synthase of a compound having the formula:



wherein:

X and Y form a five- or six-membered heterocyclic ring containing at least one nitrogen atom;

Z is a hydrogen, halogen, carbon, oxygen or nitrogen atom;

U is a carbon or nitrogen atom;

n is 0 or the integer 1;

V is a carbon or nitrogen atom;

W is a carbon or nitrogen atom;

A is in the 1- or 2-position and is a nitrogen atom, a sulfur atom, or a substituted or unsubstituted alkylene group;

Ar is an aryl or heteroaryl group having one or more rings; and

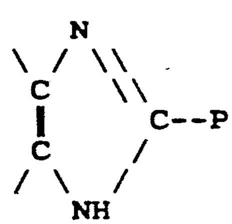
B is either (i) an oxygen or nitrogen atom, or a -CO- or $-SO_2-$ group, any of which is linked to an amino acid, aryl group or alkyl group, or (ii) a substituted or unsubstituted alkyl group,

with the proviso that, if A is sulfur, both V and W are carbon atoms.

48. The composition of claim 47 wherein said compound has a thymidylate synthase inhibition constant K_i of less than or equal to about 10^{-4}M.

49. The composition of claim 47 wherein X and Y form a heterocyclic ring selected from the group consisting of pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine and pyridazine rings.

50. The composition of claim 47 wherein X and Y form the ring:



wherein P is a lower alkyl group, an amino group or hydrogen.

51. The composition of claim 47 wherein Z is hydrogen.

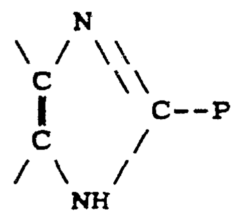
52. The composition of claim 47 wherein n is O.

53. The composition of claim 47 wherein A is substituted or unsubstituted alkylene group.

54. The composition of claim 47 wherein Ar is a phenyl or naphthyl group.

55. The composition of claim 47 wherein B is an $-SO_2$ group linked to an amino acid, aryl group or alkyl group.

56. The composition of claim 47 wherein X and Y form the ring:



wherein P is a lower alkyl group, an amino group or hydrogen;

Z is hydrogen;

U is carbon;

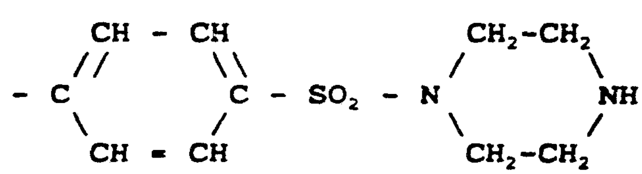
n is O;

V is carbon;

W is nitrogen;

A is methylene; and

Ar-B is selected from the group consisting of:



;

```
       CH - CH                 CH - CH
      //      \\              //      \\
-- C            C - SO2 - C            CH      ;
      \       /               \       /
       CH = CH                 CH = CH

       CH - CH                 CH - CH
      //      \\              //      \\
-- C            C - SO2 - C            C-OH    ;
      \       /               \       /
       CH = CH                 CH = CH

       CH - CH                 CH - CH
      //      \\              //      \\
-- C            C - SO2 - C            C-OCH3  ;
      \       /               \       /
       CH = CH                 CH = CH

     CH     CH
 \ /   \\ /   \\
  C      C     CH
  ‖      |      |
  CH     C      C--CH2-OH                       ;
   \   // \   //
     CH     CH

       CH - CH           COOH
      //      \\          |
-- C            C - CO-NH-CH-CH2-CH2-COOH       ;
      \       /
       CH = CH

     CH     CH
 \ //  \ /   \\
  C      C     C - O-R wherein R is H or
  |      ‖     |         alkyl, such as
  S ---  C     CH        methyl; and
          \   //
            CH

     CH     CH2-OH
 \ /   \\ /
  C      C
  ‖      |
  CH     C
   \   // \
     C      T wherein T is H or -CN.
     |
     CH2-OH
```

57. The composition of claim 47 wherein the amount is an efficacious amount.

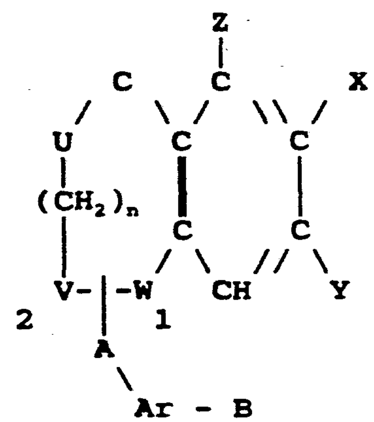
58. The composition of claim 47 in a form selected from the group consisting of forms suitable for oral, parenteral, topical, intravaginal, intranasal, intrabronchial, intraocular, intraaural and rectal administration.

59. The composition of claim 47 further comprising at least one other compound which is an antitumor agent.

60. The composition of claim 59 wherein the other compound is selected from the group consisting of mitotic inhibitors, alkylating agents, DHFR inhibitors, antimetabolites, intercalating antibiotics, enzymes, topoisomerase inhibitors or biological response modifiers.

61. The composition of claim 47 further comprising at least one other compound which is an antibacterial agent, an antifungal agent, an antiparasitic agent, an antiviral agent, an antipsoriatic agent, an antiprotozoal agent or an anticoccidial agent.

62. A therapeutic process of inhibiting thymidylate synthase comprising administering to a vertebrate host an amount effective to inhibit thymidylate synthase of a compound of the formula:



wherein:

X and Y form a five- or six-membered heterocyclic ring containing at least one nitrogen atom;

Z is a hydrogen, halogen, carbon, oxygen or nitrogen atom;

U is a carbon or nitrogen atom;

n is 0 or the integer 1;

V is a carbon or nitrogen atom;

W is a carbon or nitrogen atom;

A is in the 1- or 2-position and is a nitrogen atom, a sulfur atom, or a substituted or unsubstituted alkylene group;

Ar is an aryl or heteroaryl group having one or more rings; and

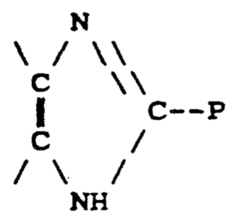
B is either (i) an oxygen or nitrogen atom or a -CO- or $-SO_2-$ group, any of which is linked to an amino acid, aryl group or alkyl group, or (ii) a substituted or unsubstituted alkyl group,

with the proviso that, if A is sulfur, both V and W are carbon atoms.

63. The process of claim 62 wherein the compound capable of inhibiting thymidylate synthase has a thymidylate synthase inhibition constant K_i of less than or equal to about 10^{-4}.

64. The process of claim 62 wherein X and Y form a heterocyclic ring selected from the group consisting of pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine and pyridazine rings.

65. The process of claim 62 wherein X and Y form the ring:



wherein P is a lower alkyl group, an amino group or hydrogen.

66. The process of claim 62 wherein Z is hydrogen.

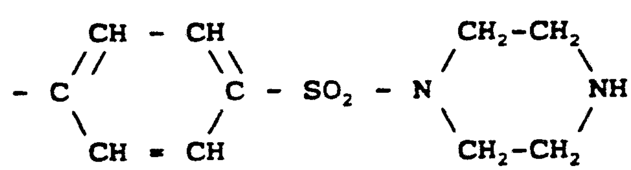
67. The process of claim 62 wherein n is 0.

68. The process of claim 62 wherein A is a substituted or unsubstituted alkylene group.

69. The process of claim 62 wherein Ar is a phenyl or naphthyl group.

70. The process of claim 62 wherein B is an $-SO_2-$ group linked to an amino acid, aryl group or alkyl group.

71. The process of claim 62 wherein Ar-B is selected from the group consisting of: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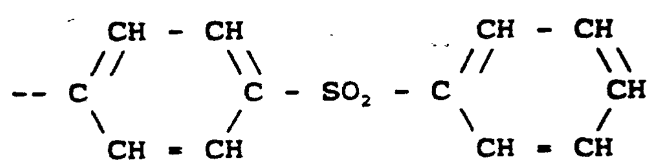
;

```
    CH - CH             CH - CH
   //     \\           //     \\
-- C       C - SO2 - C         C-OH      ;
   \      /           \       /
    CH = CH             CH = CH

    CH - CH             CH - CH
   //     \\           //     \\
-- C       C - SO2 - C         C-OCH3    ;
   \      /           \       /
    CH = CH             CH = CH

      CH      CH
   \ /  \\ /  \\
    C      C     CH
    ‖      |     |
    CH     C     C--CH2-OH              ;
     \  // \  //
      CH      CH

    CH - CH                 COOH
   //     \\                 |
-- C       C - CO-NH-CH-CH2-CH2-COOH    ;
   \      /
    CH = CH

      CH     CH
  \ //  \ /  \\
   C      C     C - O-R wherein R is H or
   |      ‖     |       alkyl, such as
   S ---  C     CH      methyl; and
           \  //
            CH

      CH    CH2-OH
  \ /  \\/
   C     C
   ‖     |
   CH    C
    \  //\
     C     T wherein T is H or -CN.
     |
     CH2-OH
```

72. The process of claim 62 wherein the compound is in a form selected from the group consisting of forms suitable for oral, parenteral, topical,

intravaginal, intranasal, intrabronchial, intraocular, intraaural and rectal administration.

73. The process of claim 62 wherein the dose of the compound is up to about 5,000 mg per square meter of the body area of the vertebrate host.

74. The process of claim 62 wherein the vertebrate host is a mammal.

75. The process of claim 74 wherein the vertebrate host is human.

76. The process of claim 62 wherein the vertebrate host is a bird.

77. The process of claim 62 wherein the compound is further characterized as producing an antiproliferative effect.

78. The process of claim 62 wherein, prior to said process, the vertebrate host harbors tumorous cells and wherein the compound is further characterized as producing an antitumor effect.

79. The process of claim 62 wherein the compound is further characterized as producing an effect selected from the group consisting of antibacterial, antifungal, antiparasitic, antiviral, antipsoriatic, antiprotozoal and anticoccidial effects.

INTERNATIONAL SEARCH REI RT

International Application No PCT/US 91/06815

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 D 471/04 A 61 K 31/435 C 07 D 487/04 //
(C 07 D 471/04 C 07 D 235:00 C 07 D 221:00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 D 471/00 A 61 K 31/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0365763 (AGOURON PHARMACEUTICALS) 2 May 1990, see claim 1 -----	1,47

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
09-01-1992	12. 02. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Maria Peis Maria Peis

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

   Although claims 62-79 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9106815 SA 51975

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 30/01/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0365763	02-05-90	AU-A- 4115389	05-04-90
		JP-A- 2174749	06-07-90

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82